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THE RELATIVE CONDUCTIVITY OF THE TISSUES IN CONTACT WITH THE HEART

Observations on Animals with Closed Chests[1]

E. LINDNER and L. N. KATZ

From the Cardiovascular Department, Michael Reese Hospital, Chicago

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We have previously shown (1) a great disparity in the ability of the tissues surrounding the heart to conduct away the electrical currents generated in it. This dissimilarity in the electrical conductivity of the cardiac contacts was considered to be of importance in determining the electrical field created in the body, and helped to explain the special value of precordial leads (2). It might be argued, however, that since these observations were made in open-chested animals under artificial respiration they did not apply to the unoperated animal in which the more intimate contact of the heart and lungs with the anterior chest wall might prove to be significant. Our previous work was, therefore, repeated in animals with chests closed during natural respiration.

Method. Sixteen dogs anesthetized with nembutal were used in this study. Throughout each experiment the animal was kept in one position upon its back. In seven, the effect of temporary opening of the chest and handling of its contents was noted as follows: After tracheotomy, a three lead control electrocardiogram was taken. The chest was then opened midsternally, artificial respiration instituted, the anterior mediastinum cleared away, and the lungs and heart manipulated. On closing the chest air tight, any residual pneumothorax was relieved and natural breathing reëstablished. The electrocardiogram was repeated for comparison with the control.

In two animals the effect on the electrical field of merely ligating all the systemic blood vessels of the heart was determined, the only circuits remaining being the coronaries and the lungs. The chest was closed and arti-

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ficial respiration was employed. No subsequent control tracings were recorded. To test whether such ligation was equivalent to complete reduction of the vessels' ability to conduct, the experiments were repeated in three other animals in which, in stages, the ligated vessels were cut close to the heart and separated. Several preliminary tests showed that additional rubber insulation between the cut ends made little difference in the results.

In the remaining experiments the heart was partially insulated with heavy rubber sheeting placed in various positions. For this purpose the chest was opened as described above, the rubber sheeting inserted at the desired location, the chest again closed and normal respiration reëstablished before the record was taken.

Five types of insulation were used:

A. In six dogs the rubber sheeting was interposed between the heart and the lungs so that the lateral surfaces of the former were insulated from the latter except at the hiluses of the lungs.

B. In three dogs the heart, lungs and inferior vena cava were insulated from the posterior paravertebral muscle mass by lining the entire dorsal half of the thoracic cavity with rubber sheeting from the diaphragm to as cephalad a region as possible.

C. In these same three dogs, posterior insulation was again effected by placing the heart in a rubber cradle so that its posterior and lateral surfaces as cephalad as possible were electrically insulated from the surrounding tissues.

D. In four dogs the heart was surrounded by rubber sheeting so that its anterior, lateral and inferior surfaces were electrically insulated.

E. Subsequently in these same four dogs the rubber sheeting was rearranged so as to cover only the anterior and lateral surfaces of the heart.

In each of these experiments records were taken: *a*, before insertion of the insulation; *b*, while the insulation was in place, and *c*, after the insulation was removed, the chest being opened temporarily between sets of tracings for the purpose of inserting or removing the rubber insulation. The voltage of the QRS complex in each lead of each record was measured, corrected when necessary for standardization and the sum of the voltage in the three leads determined. The effect of insulation was estimated by the percentage decrease in the sum of the voltage of the three leads during the insulation, considering the average of the sums of the preceding and succeeding control records in each experiment as 100 per cent. All experiments in which there was any appreciable disparity between the two controls were discarded.

Discussion of results. It was found that the "mock" operation produced a variable change. The average of the seven experiments was

+4 per cent; the range from −15 to +30 per cent in individual experiments (see table 1). This variable change we attribute to displacement of the heart during the manipulation. In these experiments it was not possible to immobilize the heart as in our previous open-chested studies (1). For this reason the effect of various types of insulation was considered in terms of the average results obtained in each type of experiment. In six experiments of types B and C, it was found that the QRS voltage decreased 34 per cent on the average (see table 3). This indicates that the posterior

TABLE 1

Effect on the sum of the QRS voltage of the three limb leads of the "mock" operation

	EXPERIMENT NUMBER							AVERAGE
	1	2	3	4	5	6	7	
% deviation from control before "mock" operation.....	+10	−10	+5	+30	+10	−5	−15	+4

TABLE 2

Effect on the sum of the QRS voltage of the three limb leads of insulation of the heart from the lungs (procedure A)

	EXPERIMENT NUMBER						AVERAGE
	1	2	3	4	5	6	
% deviation from controls.....	+10	−5	+25	0	−10	0	+4

TABLE 3

Effect on the sum of the QRS voltage of the three limb leads of insulation of the heart from the posterior muscle mass (procedure B and C)

	EXPERIMENT NUMBER						AVERAGE
	1	2	3	4	5	6	
% deviation from controls.....	−40	−40	−30	−5(?)	−50	−40	−34

and lateral surfaces of the heart, as far as they could be insulated, conduct roughly 34 per cent of the electrical field generated in the heart. The average of six experiments of type A showed that insulation of the lateral surfaces of the heart caused an increase in voltage of +4 per cent (see table 2), indicating that the lungs conduct practically none of the electricity generated by the heart. The average reduction in voltage following procedure D in our experiments was 39 per cent (see table 4). This represents roughly the proportion of the heart's currents carried to the field by way of the diaphragm and the anterior chest wall. Of this the anterior

chest wall carries away 14 per cent of the current as shown by the average reduction obtained in the four experiments of type E (see table 5). The difference, 25 per cent, represents the portion of the current conducted away by the diaphragm and its adjacent organs. In the two experiments

TABLE 4

Effect on the sum of the QRS voltage of the three limb leads of insulation of the heart from the lungs, anterior chest wall and diaphragm (procedure D)

	EXPERIMENT NUMBER				AVERAGE
	1	2	3	4	
% deviation from controls	−45	−25	−50	−40	−39

TABLE 5

Effect on the sum of the QRS voltage of the three limb leads of insulation of the heart from the anterior chest wall and lungs alone (procedure E)

	EXPERIMENT NUMBER				AVERAGE
	1	2	3	4	
% deviation from controls	−30	−10	0	−15	−14

TABLE 6

Effect on the sum of the QRS voltage of the three limb leads of ligation of the ascending thoracic aorta and venae cavae

	EXPERIMENT NUMBER		AVERAGE
	1	2	
% deviation from controls	−15	−5	−10

TABLE 7

Effect on the sum of the QRS voltage of the three limb leads of ligation and separation of the ascending thoracic aorta and venae cavae

	EXPERIMENT NUMBER			AVERAGE
	1	2	3	
% deviation from controls	−35	−50	−35	−40

performed, it was found that merely ligating the systemic blood vessels reduced the voltage by only 10 per cent (see table 6). However, when the ligated vessels were cut and separated in three other experiments, the effect was striking and in accord with the findings of Eyster et al. (3) (see table 7).

On the assumption that +4 per cent in the case of lung insulation indicates no conduction, we are able to account for 73 per cent of the heart's electrical field picked up in the limb leads as being transmitted via the lateral, posterior, inferior and anterior surfaces of the heart. The distribution while definitely a rough one, is nevertheless significant, and is shown in table 8. This variability in the electrical conductivity of the various tissues surrounding the heart is supported by the recent work of Benjamin, Landt et al. (4). In our previous open-chested experiments (1) the seemingly greater effect of insulation from the posterior muscle mass and the inconsistency of insulation from the diaphragm can be easily explained. Due to the absence of the chest wall, the diaphragm tends to move away from the heart, and it is possible that variable over-inflation of the lungs occurred during artificial respiration. The contact between the heart and diaphragm would be poorer, and as a result, the posterior muscle mass becomes by far the most important electrical conductor in

TABLE 8

Relative proportion of heart's currents transferred to the body field by the tissues adjacent to the heart

Lateral heart surfaces	0%
Anterior heart surface	14%
Inferior heart surface	25%
Posterior heart surface	34%
Total	73%
Superior heart surface (calculated)	27%

the open-chested animal. Insulation of the heart from this good conductor, therefore, had a much greater effect in these animals than in the present series with chests intact. By comparison also the effect of insulation of the inferior surface was greatly decreased in the former experiments. Actual measurement of total control voltage in both series bears this out, that of the present report being considerably greater. The large effect calculated for the superior heart surface is to be attributed to the location here of most of the large systemic blood vessels. The fact that the actual average effect is greater for the blood vessels, viz., 40 per cent, table 7, may be due to the contact of these vessels with other heart surfaces.

Our present experiments with closed thorax confirm the view previously expressed by us that the posterior muscle mass is the best electrical conductor, being approached only by the diaphragm with its adjacent viscera on the one hand and the superior mediastinum on the other. The anterior chest wall is a poorer conductor, and the lungs apparently act as almost complete insulators.

It was observed in these experiments that subtotal insulation of the heart in these various ways has an approximately equal effect on the voltage of all phases of QRS and of the P and T waves as well. The effect of insulation was greatest as a rule in lead I. The equal effect on the various complexes of the electrical cycle in each lead, regardless of where the insulation was located or what its degree, indicates that the heart itself is an excellent electrical conductor. Were this not so, an unequal change in the contour of the complexes would have been anticipated at least with some forms of insulation.

SUMMARY

Observations were made on sixteen nembutalized dogs in studying the effects of insulation of various regions around the heart on the voltage of the QRS of the standard limb-lead electrocardiograms. All animals were breathing normally with chests closed at the time records were taken. The following were the results obtained:

"Mock" operation had no appreciable effect on the strength of the peripheral electrical field of the heart.

Insulation between the lungs and the lateral surfaces of the heart produced no apparent change.

Insulation of the heart from the posterior muscle mass resulted in an approximate reduction of voltage of 34 per cent.

Insulation of the heart from the anterior chest wall reduced the voltage by 14 per cent.

Insulation of the heart from the diaphragm decreased the voltage by 25 per cent.

Ligation close to the heart of all the vessels of the larger circuit (except the coronary circuit) reduced the voltage by 10 per cent, while separating the cut ends of these vessels reduced the voltage 40 per cent.

By elimination of the other surfaces, therefore, the region immediately cephalad to the base of the heart carries roughly 27 per cent of the current generated by the heart to the body.

It is suggested that the heart itself, because of the absence of any effect on the electrocardiogram save that of decrease in voltage, must be an excellent conductor.

We are indebted to Dr. H. C. Bazett whose criticism of our original interpretation of the rôle of the systemic blood vessels in carrying away the currents generated by the heart stimulated us to further experiments.

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RENAL FUNCTION IN EXPERIMENTAL ADRENAL INSUFFICIENCY[1]

HAROLD E. HARRISON AND DANIEL C. DARROW

From the Department of Pediatrics, Yale University School of Medicine

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At an early stage in the investigation of the functions of the adrenal cortex, Marshall and Davis (1) demonstrated an impairment of renal function in adrenalectomized animals with respect to the excretion of injected creatinine and phenolsulfonphthalein. Subsequently, the observations of Loeb and co-workers (2, 3) conclusively showed a relationship between the adrenal cortex and renal function, especially as related to the maintenance of the normal concentrations of sodium and other electrolytes in the blood plasma. Harrop, Nicholson and Strauss (4) found that untreated adrenalectomized dogs retained potassium and that in these dogs the injection of adrenal cortical extract was followed by a sharp increase in urinary excretion of potassium. In line with these results we have found an increased concentration of potassium in the intracellular water of muscle as well as in the extracellular fluids of the body of adrenalectomized rats, cats, and dogs showing signs of adrenal insufficiency (5, 6). The object of the present investigation was to determine whether this potassium accumulation could be the result of disturbed renal function, and to study the physiological mechanisms involved.

The basic plan of the experiment was to determine in the normal and the adrenalectomized dog the volume of glomerular filtrate formed and simultaneously the tubular reabsorption of water and electrolytes. In the dog the plasma creatinine clearance may be used as a measure of glomerular filtration (7). As an index of the tubular reabsorption of electrolytes in relation to water, we propose to use the ratios of the concentrations of electrolytes in the urine and plasma. These ratios indicate the function of the tubules in reabsorbing water and solutes at different rates. If water and a given ion are reabsorbed to the same extent, the concentration of the ion in the urine must be the same as that in the glomerular filtrate. Therefore, the degree to which the concentration of an ion in the urine differs from that in the plasma is a measure of tubular function with respect to differential absorption of water and electrolytes.

[1] This investigation was aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

The electrolytes studied in these experiments were sodium, chloride, potassium and phosphate. Urea clearances were also determined in addition to the creatinine clearance.

PROCEDURE. Two healthy male dogs were used as the subjects of the experiments. After preliminary studies during the normal state, bilateral adrenalectomy was performed in two stages. The animals were fed a diet consisting of fresh ground beef with some milk. Since this diet contained no added sodium chloride and is high in potassium, the dogs went into a marked state of adrenal insufficiency in from three to seven days if given no treatment. Both animals were studied during repeated cycles of adrenal insufficiency followed by cure either with sodium salts alone or sodium salts in conjunction with cortical extract. The animals were permitted to go into a terminal stage of adrenal insufficiency three months and one month, respectively, after the adrenalectomy. No adrenal tissue was found at autopsy.

All studies were done at least 18 hours after the last feeding. The urine was collected by an inlying catheter, the bladder being washed with distilled water at the beginning and end of each experimental period. About two hours before the collection periods, the dogs were given a solution of creatinine by stomach tube in order to raise the concentration of creatinine in the plasma to values between 10 and 20 mgm. per cent. In the intact dog and the adrenalectomized dog treated with cortical extract, KCl or KH_2PO_4 was also given by gavage or intravenously in the amounts shown in the tables in order to raise the concentrations of serum potassium and phosphate to the levels found in untreated adrenalectomized animals. In period 10, dog A, Na_2HPO_4 was injected intravenously in order to be sure that maximal stimulation for phosphate excretion was obtained during one period of adrenal insufficiency.

In each case, the individual period of collection was approximately one hour. Blood was withdrawn under oil at the beginning and end of each period and all analyses carried out on separated serum.

The serum and urine samples were analyzed by the same methods. Creatinine was determined by the Folin method (8); urea by the Van Slyke and Kugel hypobromite method[2] (9); sodium by the Butler and Tuthill modification of the Barber and Kolthoff method (8); potassium by the method of Harrison and Darrow (10); and phosphorus by the Fiske and Subbarrow method (11). All colorimetric determinations were made with a photoelectric colorimeter of the Evelyn type (12).

The plasma clearances for the various solutes were calculated by the usual formula:

$$\text{Plasma clearance, ml./min.} = \frac{UV}{P}$$

U = concentration in urine, V = volume of urine in ml. per minute, and P = concentration in plasma. (The plasma concentration used was the average of the concentrations at the beginning and end of the collection period.)

In tables 1 and 2 are presented the essential data concerning the electrolyte excretion of dogs A and B, respectively. In table 3, the creatinine

[2] In the determination of urea in the urine ammonia was not removed, so that the urea concentrations represent actually urea plus ammonia. These results may be used for comparative purposes inasmuch as under the conditions of the experiment ammonia excretion was not stimulated. In several experiments in which the urine ammonia was determined, the amounts were too small to affect significantly the results.

and urea clearances for the same experimental periods are listed. The data are given separately for each collection period.

Since the essential information can be obtained without protocols, the various determinations were put into four categories: 1. *Normal state:*

TABLE 1

Electrolyte excretion—dog A, weight 11 kgm.

	TREATMENT	PLASMA			U/P				WATER EXCRETION	K EXCRETION
		K	Na	P	K	Na	P	Creatinine		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
					Normal					
		mM./l.	*mM/l.*	*mgm./100 ml.*					*ml./min.*	*mM./hr.*
1	NaCl, 10 gm.	4.5	148	4.4	9	1.4	3	132	0.54	1.4
2	KCl, 1 gm.	5.2	147	4.1	29	0.1	0.5	571	0.08	0.7
3	KH_2PO_4, 7.5 gm.	5.5	147	7.7	27	0.2	50	144	0.34	3.0
4	KH_2PO_4, 10 gm.	6.5	147	6.8	40	0.4	54	144	0.50	8.0
5	KH_2PO_4, 10 gm.	7.0	147	9.6	43	0.1	64	132	0.44	8.0
					Insufficiency					
6		5.9	123	6.6	8	1.2	18	114	0.33	0.9
7		6.7	130	7.8	11	0.3	14	48	0.40	1.7
8		7.5	124	10.0	10	0.2	11	37	0.38	1.7
9		8.0	130	8.2	12	0.4	13	127	0.11	0.7
10	Na_2HPO_4, 1.0 gm.*	8.4	132	14.0	10	1.2	14	28	0.64	3.0
					"Sodium" treated					
11		5.6	139	7.5	9	1.2	14	19	1.62	4.8
12		6.2	128	5.4	5	1.7	9	60	0.82	1.4
13		7.5	136	5.1	8	2.1	8	53	0.78	2.9
14		7.9	143	5.2	7	2.5	23	57	0.61	1.9
15	KCl, 0.7 gm.*	10.2	133	3.8	2	2.0	5	35	1.11	1.5
					"Sodium" plus cortical extract					
16		4.3	152	4.4	24	0.8	19	61	0.67	4.1
17		5.0	144	5.4	32	0.5	45	150	0.20	2.0
18	KCl, 1.8 gm.*	5.5	143	3.5	31	0.7	8	50	0.84	8.4
19		5.8	151	6.5	20	1.4	16	35	1.0	7.1
20	KH_2PO_4, 7.5 gm.	5.9	147	4.8	34	1.4	30	81	0.73	8.6
21	KCl, 2.3 gm.*	6.0	146	3.8	35	0.2	2	53	0.75	9.2

* Given by intravenous injection.

These studies all preceded the adrenalectomy and were done when the dogs looked well and suffered from no disturbance in salt and water balance. 2. *Adrenal insufficiency:* These periods all took place after bilateral adre-

nalectomy and from 2 to 5 days after withdrawal of parenteral injections of sodium salts and when symptoms of adrenal insufficiency were apparent. Adrenal insufficiency was deemed present when weakness and loss of appetite developed and when the concentration of serum sodium was decreased and that of urea and potassium was increased. 3. *"Sodium" treated:* Following a period of adrenal insufficiency the adrenalectomized dogs were restored to good condition by the daily intraperitoneal injection of a solution of NaCl and $NaHCO_3$ of double physiological concentration. The determinations always followed at least a period of ten days without cortical extract. The daily dose of salt used was NaCl 0.7 gram; $NaHCO_3$ 0.3 gram per kgm. The clearances were done about four hours after

TABLE 2

Electrolyte excretion—dog B, weight 15 kgm.

	TREATMENT	PLASMA			U/P				WATER EXCRETION	K EXCRETION
		K	Na	P	K	Na	P	Creatinine		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	Normal									
		mM./l.	*mM/l.*	*mgm./100 ml.*					*ml./min.*	*mM./hour*
1	KCl, 10 gm.	4.2	151	3.4	71	0.07	0.3	242	0.26	4.6
2	KH_2PO_4, 12 gm.	5.4	147	6.1	75	0.09	81	217	0.30	7.2
3	KCl, 4 gm.	4.9	146	1.7	34	0.88	0	70	0.98	9.8
	Insufficiency									
4		5.9	134	5.9	8	0.22	7	38	0.72	2.1
	"Sodium" treated									
5		4.6	141	8.3	5	1.4	19	30	1.61	2.4

the last intraperitoneal injection of NaCl and $NaHCO_3$. 4. *Extract treated:* These studies followed a period of insufficiency and were preceded by the daily injection of the same amounts of salt as in the "sodium" treated periods. However, in addition to sodium salts, the dogs received a daily subcutaneous injection of 10 cc. of cortical extract (Upjohn Company) for three days, including the day of the experiment.

The mode of regulation of electrolyte excretion in the normal dog and the disturbances found following adrenalectomy are clearly demonstrated by study of the concentrations in urine of sodium,[3] potassium and phosphorus as compared to the concentrations of these ions in plasma.

[3] Excretion of chloride was so similar to that of sodium that the results for chloride will neither be reported nor discussed.

TABLE 3

Creatinine and urea clearances in the normal and adrenalectomized dog

DOG A				DOG B			
Period	Plasma urea N	Clearance		Period	Plasma urea N	Clearance	
		Creatinine	Urea			Creatinine	Urea
Normal							
	mgm./100 ml.	*ml./min.*	*ml./min.*		*mgm./100 ml.*	*ml./min.*	*ml./min.*
1	16	71	42	1	9	63	41
2	14	44	25	2	10	64	45
3	17	49	26	3	8	69	60
4	27	72	38				
5	8	58	33				
Average.....		58	33			65	49
Insufficiency							
6	31	38	18	4	69	27	8
7	81	19	8				
8	105	14	4				
9	63	14	2				
10	82	18	7				
Average.....		21	8				
"Sodium" treated							
11	86	31	18	5	12	49	39
12	18	49	32				
13	22	41	26				
14	23	35	21				
15	13	39	30				
Average.....		37	25				
Extract treated							
16	10	41	25				
17	14						
18	9	41	28				
19							
20	16	59	35				
21	10	40	32				
Average.....		45	30				

In the normal animal during the fasting state, the U/P ratios for sodium are considerably less than 1, indicating almost complete reabsorption of sodium by the renal tubules. During period 1 for dog A, when sodium

salts were administered reabsorption of sodium was decreased as shown by a U/P ratio greater than 1.

The administration of potassium or phosphate salts to the normal dogs results in the excretion of these ions at high concentration in the urine. There is, however, an apparent limiting U/P ratio which is not exceeded. The maximum U/P ratios for potassium are 43 and 75 for dogs A and B, respectively. In experiments on human subjects, Keith and Binger (13) found that potassium was concentrated in the urine to about 50 times the plasma concentration following the ingestion of large amounts of potassium salts. In the present experiments the U/P ratios for potassium and phosphorus are always lower than those for creatinine, indicating that some reabsorption of these ions always occurs in the renal tubules. Because there is a limiting concentration of potassium in the urine, the actual potassium excretion may vary with the urine volume as shown in the last two columns of tables 1 and 2. The maximum U/P values for phosphate are somewhat greater than those for potassium. Also, at low concentrations of phosphate in the plasma, the U/P ratios may be much less than 1. In the case of potassium U/P values of less than 1 have not been observed.

Following bilateral adrenalectomy, as adrenal insufficiency develops the concentration of plasma sodium decreases and the concentration of potassium increases to values considerably above the normal level of about 4 mM. per liter. The plasma phosphorus also rises to unusually high concentrations. These changes are the result of disturbances in the excretion of electrolytes in the urine.

The concentrations of sodium in the urine are higher in the adrenalectomized dog than in the normal dog under similar experimental conditions. In two of the five experiments on dog A, despite the falling concentration of sodium in the plasma, sodium continues to be excreted in the urine at a concentration approximately equal to that of the plasma. The reabsorption of sodium is definitely limited. In the experiments in which the U/P ratios for sodium are less than one, the creatinine clearances are found to be greatly reduced (table 3). This would indicate that more complete reabsorption of sodium can occur in the renal tubules of the adrenalectomized dog if glomerular filtration is greatly reduced, thus increasing the time during which absorption may take place in the tubules. However, even under conditions of diminished glomerular filtration the U/P ratios for sodium in the adrenalectomized dogs are still greater than those of the normal animals. Moreover, normal animals depleted of sodium may excrete urine containing practically no sodium whatsoever (table 6).

The maximum concentrations of potassium and phosphorus in the urine, on the other hand, are much lower in adrenal insufficiency than those found in the normal animal with comparable concentrations of these ions in the plasma. The limiting U/P ratio for potassium is approximately

10 in the adrenalectomized dog as compared to 40 to 75 for the normal dog. Inasmuch as the volume of urine is unchanged, the excretion of potassium is much lower during adrenal insufficiency than in the normal state.

The disturbances in electrolyte excretion in adrenal insufficiency are complicated by the coincident finding of greatly reduced creatinine clearances which indicate a reduction in glomerular filtration. The abnormality of potassium excretion is not, however, dependent upon the change in glomerular filtration and this will be more clearly shown in the experiments in which the dogs are treated with sodium salts alone and with sodium salts in conjunction with adrenal cortical extract.

The daily intraperitoneal injection of a hypertonic solution of sodium chloride and sodium bicarbonate results in an increase of the creatinine and urea clearances from the low levels found in adrenal insufficiency (table 3) but does not restore to normal the renal excretion of electrolytes. The concentrations of plasma potassium in the "sodium treated" experiments are uniformly high even though no extra potassium salts were given on the day of the experiment except for period 15, dog A. In these experiments the failure of the renal tubules to concentrate potassium in the urine is evident, the U/P ratios being uniformly below 10. The urine output, however, of the "sodium treated" dogs is considerably greater than that of the untreated animals in adrenal insufficiency. For that reason the potassium excretion is increased following the treatment with sodium salts even though the concentration of potassium in the urine remains low.

The objection might be raised that diuresis produced by the treatment with sodium salts is enough to prevent potassium from being excreted at high concentrations in the urine. The results obtained in the cortical extract experiments effectively answer this point. In these experiments dog A was given the same dosage of sodium salts as in the experiments of group III, but in addition was treated with adrenal cortical extract (Upjohn) in a dosage of 10 ml. daily for two days prior to each experiment. The urine volumes in the cortical extract experiments are approximately the same as those of the "sodium treated" group. The creatinine clearances are also approximately the same. The striking difference is the greatly increased concentration of potassium in the urine following treatment with cortical extract, the U/P ratios ranging between 20 and 35. The potassium excretion is high because of the combination of a large urine volume plus a high concentration of potassium in the urine.

The effect of the adrenal cortical extract is also evidenced by the lessened concentration of sodium in the urine following injection of cortical extract as compared to the concentrations found following treatment with sodium salts alone.

The rapidity with which the effect of injected adrenal cortical hormone on renal function is manifested is shown by the following experiments.

Successive hourly collections of urine with simultaneous blood samples were taken, first while the animal was in a state of adrenal insufficiency and then subsequent to the injections of 20 ml. of adrenal cortical extract alone or of 20 ml. of adrenal cortical extract plus the intravenous injection of a hypertonic solution of sodium salts. The results of the two experiments are shown in table 4. Within one hour after the injection of the extract alone there is noted an increased urinary concentration of potassium and phosphorus and a decreased concentration of sodium. There is no change in the creatinine and urea clearances. Following injection of both

TABLE 4

The effect of adrenal cortical extract upon the relative concentrations of potassium and sodium in the urine of the adrenalectomized dog A

PERIOD	PLASMA			U/P			CREATININE CLEARANCE	URINE VOL.
	K	Na	P	K	Na	P		
	I							
	mM./l.	*mM./l.*	*mgm./100 ml.*				*ml./min.*	*ml./min.*
Insufficiency	6.2	130	7.7	11	0.33	14	19	0.4
12:37 p.m. 20 ml. adrenal cortical extract								
1:30–2:30	5.7	132	9.0	19	0.07	20	14	0.15
2:30–3:30	5.7	132	9.0	19	0.03	17	18	0.30
Overnight	5.7	130	6.4	19	0.04	34	—	0.28
	II							
Insufficiency	8.4	132	8.2	12	0.36	12	14	0.1
1:10 p.m. 2.5 gm. $NaHCO_3$ and 7.0 gm. NaCl in 100 ml. water intravenously 20 ml. adrenal cortical extract								
2:00–3:00	6.8	153	6.1	11	1.6	13	38	2.0
3:00–4:00	5.8	151	6.4	20	1.4	16	35	1.6
Overnight	5.1	144	5.4	32	0.45	46	30	0.2

cortical extract and a hypertonic solution of sodium chloride and bicarbonate, a progressive increase in potassium and phosphorus concentrations in the urine is noted, starting within two hours after the extract was injected. These changes in concentration of potassium and phosphorus in the urine following adrenal cortical extract are not to be explained by changes in urine volume. In one period of adrenal insufficiency the U/P ratio for potassium is 12 with a urine volume of only 0.1 ml. per minute. Following the administration of adrenal cortical extract plus sodium salts the U/P ratio for potassium increases despite a marked increase in urine volume.

The constant finding of a relatively low concentration of potassium in the urine of the adrenalectomized dog is not associated with a low total concentration of electrolytes in the urine. In table 5 is given the sum of the concentrations of sodium and potassium in the urine. If groups III and IV are compared, in which the treatment was the same except for the administration of adrenal cortical extract in the experiments of group IV, it is apparent that the concentrations of sodium plus potassium are of the same order of magnitude, although the concentration of potassium alone is low in the experiments of group III and high in those of group IV. The failure of the kidneys of the adrenalectomized dog to excrete a urine of high potassium concentration cannot, therefore, be due to an inability of the renal tubules to do osmotic work, i.e., reabsorb water against an increasing concentration of solutes in the urine. The disturbance of function involves the differential absorption of sodium, potassium, phosphorus and perhaps other ions. This selective reabsorption is necessary to maintain normal concentrations of electrolytes in the body fluids.

The decreased creatinine and urea clearances found in adrenal insufficiency are probably secondary to the disturbances in electrolyte excretion.

TABLE 5

Concentrations of sodium plus potassium in urine of normal and adrenalectomized dog

PERIOD	Na + K
	Mm./l
I Normal	167–323
II Insufficiency	98–231
III "Sodium" treated	184–400
IV Cortical extract	222–389

In adrenal insufficiency, the creatinine clearance drops to as low as 25 per cent of the average normal value (table 3). The urea clearances decrease with the creatinine clearances but to an even greater extent, so that during the most severe stages of adrenal insufficiency studied the urea clearances are as low as 7 to 12 per cent of the normal. This disproportionate drop in urea clearance may be explained as being due to increased urea reabsorption which is believed to occur at diminished rates of glomerular filtration (14). It is not dependent on decreased volume of urine since it is observed with urine outputs of 0.4 to 0.7 ml. per minute, which are as great as those found in the control experiments in the unoperated dogs.

When the concentration of sodium in the extracellular fluid is partially restored by means of injections of hypertonic solutions of sodium chloride and sodium bicarbonate, the creatinine and urea clearances rise to about 65 to 75 per cent of the normal values and the normal ratio between the creatinine and urea clearances is restored. Treatment with cortical extract

in addition to the sodium salts results in no significant further increase in the creatinine and urea clearances. However, inasmuch as the creatinine clearances in these experiments are less than the maximum normal values it is possible that treatment with larger amounts of extract might show a definite increase in the creatinine clearance.

The effect of treatment with sodium salts upon the rate of glomerular filtration is surprisingly rapid. In one experiment, the creatinine and urea clearances rose from 14 and 4 ml. per minute, respectively, to 31 and 18 ml. per minute within one hour following the injection of a hypertonic solution of sodium chloride and sodium bicarbonate. This rapid increase in the creatinine and urea clearances following the restoration of

TABLE 6

The creatinine and urea clearances of an intact dog depleted of sodium by the intraperitoneal injection of glucose

DATE	PERIOD	PLASMA		CREATININE CLEARANCE	UREA CLEARANCE	U/P	
		Na	K			Na	K
		Mm./l.	*Mm./l.*	*ml./min.*	*ml./min.*		
March 28	Normal*	148	5.4	65	49	0.1	75
April 8	Sodium depletion, 24 hr.	131	4.5	20	8	0	11
April 11	Sodium depletion, 96 hr.†	133	5.1	38	18	0.02	70
April 15	Saline treated	148		73	41		

* 12.0 grams KH_2PO_4 given p.o., 90 minutes prior to collection period.

† 12.0 grams KH_2PO_4 given p.o., 120 minutes prior to collection period.

Protocol of experiment. April 7. Fifteen hundred milliliters 5 per cent glucose solution injected intraperitoneally at 10 a.m.; 1300 ml. ascitic fluid removed at 4 p.m. Given NaCl free diet in form of washed hamburger.

April 8. Dog very weak, refuses to eat. Clearance experiment.

April 11. Dog continues weak although more active than three days previously. Refuses to eat. Clearance experiment.

April 12. Five grams NaCl given in drinking water. Given NaCl containing diet. Food taken well.

April 15. Normal appearance, vigorous. Clearance experiment.

the normal concentrations of sodium and chloride in the plasma and interstitial fluids indicates that the reduced creatinine clearance in adrenal insufficiency is secondary to the effects of the deficit of these ions in the body. This concept is confirmed by experiments in which animals with intact adrenals are depleted of sodium. The results of an experiment of this type are shown in table 6. An unoperated dog was depleted of sodium by the intraperitoneal injection of glucose solution, as described by Darrow and Yannet (15), and removal of the solution remaining in the peritoneal cavity after five hours.

There is a marked fall in the creatinine and urea clearances following sodium depletion in the intact dog. The urea clearance is decreased to

a much greater extent than is the creatinine clearance, just as in the adrenalectomized dog. Restoration of the body sodium is followed by a return to normal of both the creatinine and the urea clearances. Similar results have been reported by McCance and Widdowson (16) in experiments on human subjects depleted of sodium and chloride by profuse sweating.

Although the existing evidence warrants the assertion that in the normal dog the creatinine clearance may be used as an index of glomerular filtration, there may be some doubt about the validity of applying the findings in the normal dog to the adrenalectomized dog. For this reason, simultaneous inulin and creatinine clearances were determined in dog B, both during adrenal insufficiency and following treatment with sodium salts. If the inulin and creatinine clearances are the same, it is justifiable to assume that both are excreted by filtration through the glomeruli without appreciable reabsorption in the tubules (7). The results of the inulin and creatinine clearances are shown in table 7. In the experiment performed the results indicate that the creatinine clearances in the adrenalectomized dog may be used as a measure of glomerular filtration.

TABLE 7

Inulin and creatinine clearances in adrenal insufficiency

PERIOD	INULIN CLEARANCE	CREATININE CLEARANCE	UREA CLEARANCE
	ml./min.	*ml./min.*	*ml./min.*
I Normal	—	65	49
II Adrenal insufficiency	24	27	8
III "Sodium" treated	44	49	39

Discussion. The excretion of substances in the urine depends mainly upon two aspects of renal activity—filtration through the glomeruli and the degree of subsequent reabsorption in the tubules. The concentrations of electrolytes in the body fluids are normally maintained relatively constant by the selective action of the renal tubules in controlling the reabsorption of ions from the glomerular filtrate. During the process of differential reabsorption of water and electrolytes in the renal tubules, high concentration gradients between tubular urine and blood plasma may be developed.

In the adrenalectomized dog not given cortical extract, there is a striking impairment of that function of renal tubule cells concerned with the differential reabsorption of electrolytes. The reabsorption of sodium is limited, so that the concentration of sodium in the urine is not reduced below that of the plasma except when the rate of glomerular filtration is greatly reduced. Conversely, potassium and phosphorus are reabsorbed to a much greater extent in the renal tubules of the adrenalectomized dog than in the normal animal studied under comparable conditions. This

increased reabsorption may be explained as a failure of the renal tubules to maintain a sufficiently high concentration gradient between the tubular urine and the blood plasma with respect to these ions.

It is evident that the volume of urine excreted is an important factor in determining the electrolyte excretion in the adrenalectomized dog. Increased rates of urine excretion permit an increased excretion of potassium in the urine but also are associated with a greater loss of sodium. The administration of sodium salts to the adrenalectomized animal not only replaces the sodium lost but may produce an increased excretion of potassium due to the augmentation of urine volume. The experiments reported also explain the observation that the adrenalectomized dog can be maintained more readily on a diet low in potassium than on a high potassium diet when given no adrenal cortical extract (19).

The concentrations of electrolytes in the body fluids of the untreated adrenalectomized dog are not maintained at normal levels as a result of the disturbed function of the renal tubules. The decreased rate of glomerular filtration found in adrenal insufficiency is, in large part at least, secondary to the altered concentrations of electrolytes in the body fluids, especially the deficit of sodium in the extracellular water. As a result of the lowered concentration of sodium in the extracellular fluids of the body, there is a shift of water from the extracellular to intracellular spaces, leading to a diminished volume of extracellular fluid (5). This depletion of the extracellular fluid volume with consequent decrease in plasma volume can explain the diminished blood pressure found in the intact dog depleted of sodium (17) and in the adrenalectomized dog (18). In the adrenalectomized dog factors other than the shift in body water are probably involved in the circulatory disturbances present. There may be changes in the muscular tone of the vascular system which contribute to the final state. The changes in glomerular filtration in the adrenalectomized dog and in the intact dog depleted of sodium can be explained most readily as the result of diminished blood pressure in the glomerular capillaries. The decrease in glomerular filtration is probably an important feature of the terminal stage of adrenal insufficiency.

The retention of urea in adrenal insufficiency can be adequately explained by the decreased rate of glomerular filtration together with an increased reabsorption of urea which is secondary to the reduced glomerular filtration. Adequate treatment of the adrenalectomized animal with sodium salts therefore results in reduction of the concentration of urea in the blood because of the increased rate of glomerular filtration.

SUMMARY

Following adrenalectomy in the dog, there is marked disturbance of renal function of a specific type. The primary change is a failure of the renal tubules to maintain the necessary concentration differences between

urine and plasma with respect to certain ions. The disturbance in tubular function is shown by the failure to reabsorb sodium adequately from the glomerular filtrate at a time when the concentration of sodium in the plasma is low, and secondly by the failure to excrete potassium, phosphate and perhaps other ions at high concentration when these ions are abnormally concentrated in blood plasma. These disturbances in tubule function explain the decrease in plasma sodium and increase in plasma potassium and phosphate found in the adrenalectomized dog. Secondarily, as a result chiefly of the loss of sodium and chloride from the body, a diminished rate of glomerular filtration occurs which contributes to the final stage of disturbed renal function in adrenal insufficiency.

The administration of sodium salts to the adrenalectomized dog improves renal function by replacing the deficits of sodium and chloride in the body and by increasing the urine volume. However, the complete restoration to normal of the renal function of the adrenalectomized dog can be accomplished only by the administration of adrenal cortical extract.

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HYPOPHYSEAL AND ADRENAL INFLUENCE ON RENAL FUNCTION IN THE RAT[1]

E. L. COREY, H. SILVETTE[2] AND S. W. BRITTON

From the Physiological Laboratory of the University of Virginia Medical School

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There is considerable evidence that both the hypophysis and the adrenal cortex are importantly related to the homeostatic mechanism governing fluid metabolism in the body. In a recent study on the effects of cortico-adrenal extract administration on renal function, Silvette and Britton (1938) have advanced the hypothesis that "in the excretion of water and sodium chloride by the kidney, the diuretic hormone of the adrenal cortex acts in physiological antagonism to the antidiuretic hormone of the posterior lobe of the pituitary." Normal function of both hormones was shown to be necessary for the proper maintenance of sodium chloride and water balance. The present study was undertaken as a further test of this hypothesis.

About 300 individual metabolism experiments were carried out, the rat being employed as a test animal. The experimental period was limited to 24 hours with water supplied *ad lib.* from graduated drinking tubes. Urine was collected under toluene in graduated cylinders, and urinary chloride determined by the Volhard titration (Peters and Van Slyke, 1932). All animals were fasted during the experimental period. For initial tests the operated animals were placed in the metabolism cages immediately on recovery from the anesthetic ("Vinethene," Merck[3]); in cases in which repeated determinations were made on the same animals, the rats were removed to feeding cages for at least 24 hours before succeeding tests in the metabolism chambers.

The results are presented in brief form in table 1. Averages were employed in plotting figures 1, 2 and 3. All data obtained were subjected to biometric analysis and significant differences are noted in the text.

Hypophysectomy. Three immediate effects of hypophysectomy were noted.

[1] Grateful acknowledgment is made of aid received from the Rockefeller Foundation.

[2] E. R. Squibb and Sons Scientific Fellow in Physiology.

[3] "Vinethene" (vinyl ether—$(CH_2 \cdot CH)_2O$) was furnished through the generosity of Merck and Co. This anesthetic was particularly suited to the present study since, due to its high volatility, recovery from its effects was extremely rapid.

1. *Polyuria.* It was found, in confirmation of the reports of Richter (1936) and others, that hypophysectomized rats might exhibit *a*, normal urine output, or either *b*, a mild, or *c*, severe polyuria which was in all cases, however, transitory in nature. All three types of response were observed in our series, but the average urine output in all was found to be much greater than that seen in control and other experimental groups (tables 1 and 2; fig. 1). The increase in urine output usually took place within the first hour after operation. Return to normal urinary excretion

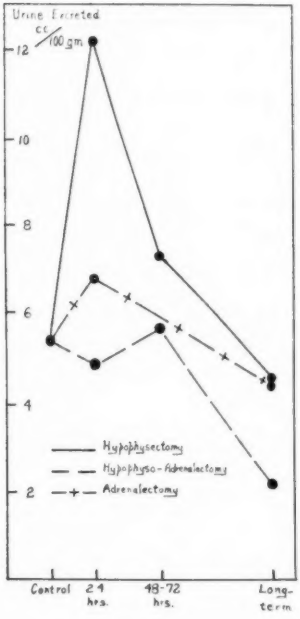

Fig. 1

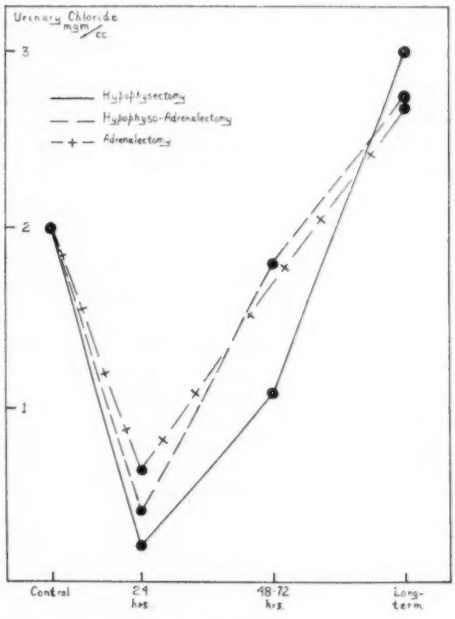

Fig. 2

Fig. 1. Urine secretion after hypophysectomy and other operations on the rat.
Fig. 2. Urinary chloride levels after hypophysectomy and other operations on the rat.

did not occur until 4 to 6 days later. Recurrence of the initial polyuria, as reported by Ranson *et al.* (1938) in the cat, was not observed.

2. *Polydipsia.* An abnormally increased water intake followed the onset of polyuria by several (2 to 6) hours. Polydipsia was therefore viewed as a secondary effect of pituitary ablation, due to excessive water loss through the kidneys which immediately followed hypophyseal extirpation.

3. *Hypochloruria.* The urine collected during the first 24 hours after

TABLE 1

Water intake and renal secretion under various conditions in the rat

	CONDITION	TIME AFTER OPERATION	NO. OF CASES	24-HOUR PERIOD		
				Water intake	Urine output	Urinary chlorides
				cc. per 100 gms. body wt.	*cc. per 100 gms. body wt.*	*mgm./cc.*
Group A (normal rats)		—	52	7.1	5.4	2.00
Group B (primary operations)	Hypophysectomized	24 hrs.	27	13.6	12.2	0.19
		48–72 hrs.	23	8.3	7.3	1.09
		Over 8 days	23	5.0	4.6	3.05
	Hypophyso-adrenalectomized	24 hrs.	10	6.6	4.9	0.43
		48–72 hrs.	12	6.4	5.7	1.81
		6–10 days	6	3.9	2.2	2.76
	Adrenalectomized	24 hrs.	18	8.9	6.8	0.65
		4–10 days	44	6.1	4.5	2.77
Group C (extract-treated rats)	Adrenalectomized. 3 cc. C.A.* extract 4 times daily	24 hrs.	6	12.0†	10.2	1.89
	Hypophysectomized. 1 cc. C.A. extract per 100 gms. every 6 hrs.	24 hrs.	10	11.0	11.3	0.22
	Hypophysectomized. 5 u. post. pituitary sol. every 4 hrs.	24 hrs.	11	1.7	2.1	5.25
	Adrenalectomized. 5 u. post. pituitary sol. every 4 hrs.	24 hrs.	6	1.4	1.2	5.89
Group D (control operations)	Sham hypophysectomy	24 hrs.	4	2.3	1.4	2.47
		96 hrs.	4	2.6	1.7	2.12
	Sham adrenalectomy	24 hrs.	6	9.6	7.3	1.27
		96 hrs.	6	9.1	9.3	1.33
	Left adrenal removed	24 hrs.	6	6.4	4.3	1.58
		96 hrs.	6	7.1	7.2	2.03
	Splenectomized	24 hrs.	6	5.0	3.1	2.65
	Thyroidectomized	24 hrs.	4	3.7	3.1	2.69
	Left kidney removed	24 hrs.	12	4.0	3.5	2.36

* "C.A. Extract" refers to cortico-adrenal extract prepared in this laboratory by a modified Swingle-Pfiffner technique (Britton and Silvette, 1931).

† By intraperitoneal injection.

operation showed a marked decrease in chloride content per cubic centimeter when compared with normal values. This condition was observed in all hypophysectomized animals, whether diuresis were present or not (table 2). The constancy of this finding in contrast to the irregularity of the extent of the polyuria, as noted above, led to the thought that urinary chloride concentration might prove the more reliable criterion of hypopituitarism in the rat.

TABLE 2

Urine output and urine chloride content of hypophysectomized rats during the first 24 hours after operation

A. SHOWING NORMAL URINE OUTPUT			B. SHOWING INCREASED URINE OUTPUT			C. SHOWING DEFINITE POLYURIA		
Rat no.	Urine excreted	Urinary chloride	Rat no.	Urine excreted	Urinary chloride	Rat no.	Urine excreted	Urinary chloride
	cc. per 100 gms. body wt.	*mgm./cc.*		*cc. per 100 gms. body wt.*	*mgm./cc.*		*cc. per 100 gms. body wt.*	*mgm./cc.*
1	4.4	0.20	4	7.0	0.37	6	24.8	0.29
2	5.3	0.36	5	7.4	0.16	8	31.0	0.24
3	3.7	0.16	9	7.3	0.15	14	11.9	0.09
7	5.4	0.19	12	8.3	0.11	15	11.2	0.16
10	5.2	0.61	13	6.7	0.12	16	16.0	0.14
11	5.8	0.21	18	8.2	0.12	17	23.0	0.23
			19	8.2	0.12	20	27.3	0.09
			26	7.3	0.13	21	15.9	0.13
						22	18.9	0.10
						23	14.0	0.09
						24	11.6	0.22
						25	19.6	0.16
						27	24.1	0.18
Average.....	5.0	0.29		7.6	0.16		19.2	0.16

Fifty-two normal rats showed an average urine excretion of 5.4 cc. per 100 grams of body weight, and a urinary chloride content of 2.00 mgm. per cc. This may be contrasted with the averages for the entire group of hypophysectomized rats listed above—urine excretion, 12.5 cc. per 100 grams; urinary chloride, 0.19 mgm. per cc.

The hypochloruria was found to be transitory, however, and urine chloride levels approaching the normal were observed from 3 to 6 days following pituitary removal. Subsequently the chlorides were maintained at a definitely heightened level as compared with controls. During the present study attention was directed principally toward the investigation of chloride changes during the first 24 hours, when hypochloruria was found to be most marked.

That the lowered chloride concentration of the urine during the first day after hypophysectomy could not be attributed to mere operative trauma or

shock was indicated, moreover, by the results of control operations. For such control purposes, 8 rats were operated on in a manner identical with that of hypophysectomy with the exception of ablation of the gland. Six animals were splenectomized, 4 thyroidectomized, and the left kidney was removed in 12 additional rats. All of these animals exhibited normal urinary chloride levels.

That the transitory hypochloruria could not be accounted for in terms of urinary dilution was quite evident. As noted above, some of the hypophysectomized rats did not exhibit polyuria, but none failed to show a considerable reduction in urinary chloride concentration (normal urine chlorides in 52 cases was 2.0 mgm. per cent; hypophysectomized—27 cases —0.19 mgm. per cent).

Adrenalectomy and combined operations. Both partially and completely adrenalectomized and also hypophyso-adrenalectomized rats exhibited no definite polyuria. Moreover, water intake in these animals showed no significant departure from the normal. Nevertheless, urinary chloride concentration was immediately reduced after operation in all cases and this persisted for 2 to 3 days, paralleling the changes observed after hypophysectomy (see fig. 2). Also, as in the hypophysectomized animals, increased concentration of urinary chlorides was observed in rats surviving more than 72 hours.

Averages of all these series showed no statistical differences (fig. 2). On the other hand, variation in the curves from those obtained from sham-operated controls was sufficiently great to demonstrate their definite departure from the normal. It appeared that these changes following hypophysectomy, adrenalectomy and hypophyso-adrenalectomy represented significant alterations in chloride metabolism, in the same direction and of approximately equal extent.

Pituitary and cortico-adrenal extract effects. A consideration of the above led to the belief that the urinary chloride reductions observed might be attributed to hypofunction (or absence) of either the hypophysis or adrenal cortex. Thus, adrenal extirpation might lead indirectly to chloride reduction by bringing about pituitary hypofunction, or hypophyseal ablation might conceivably lead to similar chloride changes through elimination of cortico-adrenotropic hormone and subsequent adrenal insufficiency.

In an attempt to analyze the possibilities, hypophysectomized and also adrenalectomized rats were injected after operation with either cortico-adrenal extract (1 cc. every 6 hours[4]) or post-pituitary solution (Squibb[5]—5 units every 4 hours).

Cortico-adrenal extract administration did not prevent urine chloride

[4] Each cubic centimeter of extract injected represented 80 grams of fresh glands. This is equivalent to twice the usual strength we have employed.

[5] Posterior Pituitary Solution, generously furnished by E. R. Squibb and Sons.

decrease in hypophysectomized rats. Hence the fall in chlorides during the first 24 hours after hypophysectomy was apparently not due to functional inactivation of the adrenal cortex. When injected into adrenalectomized rats, however, cortico-adrenal extract maintained the urinary chlorides at approximately control values. The latter experiment served to assay the extracts used and gave evidence of their potency. However the possibility that some factor originally present in the cortex might be eliminated in the process of refinement of the extracts should be considered. In contrast, the injection of pituitary solution into hypophysectomized or adrenalectomized rats resulted in each case in concentration of urinary chlorides, during the first 24 hours, to more than double the normal, preoperative level (fig. 3).

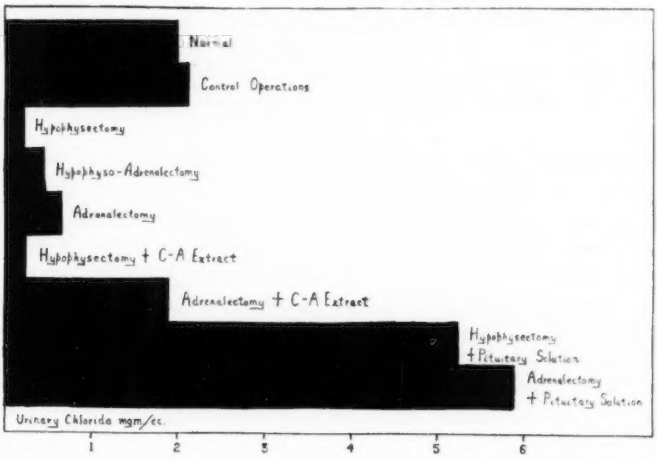

Fig. 3. A comparison of urinary chloride secretion in the rat under different conditions.

The foregoing results indicated that the hypochloruria which immediately follows hypophysectomy or adrenalectomy was attributable to lack of an hypophyseal principle elaborated by the posterior lobe. The effects of adrenalectomy on urine chloride secretion might thus be accounted for on the basis of suppression (or depression) of hypophyseal function in the absence of the adrenal cortex.

Discussion. Water intake and urine output determinations in our experiments gave results in keeping with reports by previous observers (Richter, 1933, 1934, 1936; Pencharz *et al.*, 1936; Aschner, 1912; Ranson *et al.*, 1938; Ingram and Fisher, 1936). It should be pointed out, however, that the double operation of hypophyso-adrenalectomy resulted in no

significant alterations from the normal, indicating strongly an antagonistic action between hypophyseal and cortico-adrenal secretions as regards fluid metabolism—i.e., absence of the adrenals completely counteracted the diuretic tendency which commonly followed simple pituitary ablation. These results confirm the findings of Ingram and Winter (1938) on the cat, and support the contentions of Silvette and Britton (1938) referred to above.

The abnormally increased concentration of chlorides in the urine of longer-surviving animals is in conformity with available knowledge. Thus, it has been shown that such concentration occurs some days after adrenalectomy (Silvette and Britton, 1933), and since cortico-adrenal degeneration is well known to follow hypophysectomy (Smith, 1930; Cutuly, 1936), a similar result might be expected to follow several days after pituitary ablation. On the other hand, we are confronted with significant reductions in chloride concentration during the first 24 hours following adrenalectomy as well as after hypophysectomy, in which cases reduction of chlorides might be expected concomitant with increased urine output.

The immediate effect of hypophyso-adrenalectomy may perhaps be explained on the basis of sudden release of the kidney from the effects of the post-pituitary anti-diuretic principle, allowing the more slowly-metabolized cortico-adrenal hormone (probably present to some extent in the blood and tissues for a day or so after adrenal removal) to exert its diuretic action. The immediate effect of adrenalectomy is more difficult to explain. It may be looked on as an effort in the direction of homeostasis on the part of the pituitary, or as a direct and immediate depression of pituitary function following adrenalectomy. Cytological changes in the pituitary have been described following adrenalectomy (Banting and Gairns, 1926; Hartman *et al.*, 1927), and the identity of the results obtained following either pituitary or adrenal removal, or both (in contrast to control operations), strongly suggest the latter view.

Regulation of the chloride output of the kidney has been referred by various authors to either pituitary or hypothalamic function. The experiments here reported distinctly favor the involvement primarily of some pituitary factor, and a secondary influence of the cortico-adrenal tissues. It is well to remember this in view of the fact that in the present-day treatment of Addison's disease, sodium chloride administration has been greatly emphasized. It is an obvious possibility that in adrenal affections sodium chloride and water balance may be influenced only indirectly or in correlation with disturbances of the hypophyseal-hypothalamic mechanisms.

SUMMARY

Hypophysectomy in the rat resulted in an immediate polyuria and polydipsia (80 per cent of cases) which subsided after 4 to 6 days.

Following simultaneous removal of the pituitary and adrenal glands no such effects were observed. This is interpreted as evidence of the antagonistic action of the secretions of the posterior pituitary lobe and the adrenal cortex on fluid metabolism.

In all cases, hypophysectomy, adrenalectomy, and hypophyso-adrenalectomy resulted in reduction of urinary chloride content. Concentration to abnormally high levels occurred, however, a few days later.

Injections of post-pituitary solution into hypophysectomized or adrenalectomized rats prevented the reduction of urinary chloride concentration. This material was effective, indeed, in raising the urinary chloride content two or three times above the normal value in such operated animals.

Cortico-adrenal extract administration failed to prevent hypochloruria in hypophysectomized rats, but was effective on adrenalectomized animals.

These experiments suggest that lack or deficiency of the post-pituitary hormone explains the hypochloruria following hypophysectomy or adrenalectomy. The results indicate a primary or direct involvement of the post-pituitary (or hypothalamic) mechanism in the regulation of salt and fluid balance of the body. An apparently indirect action on salt metabolism through the adrenal cortex has also been demonstrated.

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THE PASSAGE OF SORBITOL FROM THE BLOOD INTO THE AQUEOUS HUMOR AND CEREBROSPINAL FLUID[1]

LAWRENCE ROSNER AND JOHN BELLOWS

From the Departments of Biochemistry and Ophthalmology, Northwestern University Medical School, Chicago, Ill.

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In a previous report by Bellows, Puntenny, and Cowan (1), it has been shown that sorbitol administered intravenously causes a marked reduction in tension in individuals with an ocular hypertension. This substance has also been used to reduce high intracranial pressure. West and Burget (2), in experiments on dogs, have found sorbitol to be a more effective diuretic than sucrose. On the basis of comparative molecular weights of these two substances, this may be expected, since sucrose has 1.88 times the molecular weight of sorbitol and a proportionately smaller osmotic pressure. Strohm (3) has also recognized sorbitol to be active as a diuretic.

The principle of raising the blood osmotic pressure to reduce the intraocular and intracranial pressures has been employed for some time, but a serious objection to osmotic agents such as glucose was that after an initial fall, the pressure in the cranial cavity or eye rose again, generally to a point higher than the original level. This is explainable on the basis of ready passage of the agent from the blood into the aqueous humor and into the spinal fluid, so that as the glucose is removed from the blood a point may be reached where the glucose content of the aqueous and spinal fluid is higher than that of blood, and passage of water from these fluids to the blood is then reversed. It was, therefore, desirable to find some osmotic agent which did not readily pass into the aqueous humor and cerebrospinal fluid. Masserman (4) determined that sucrose entered the spinal fluid in but relatively small amounts. Since sorbitol appears to be superior to sucrose as an osmotic agent, and since its solutions in high concentrations are less viscous and therefore more easily injected than those of sucrose, we undertook to determine to what degree sorbitol in the blood would pass into the aqueous humor and cerebrospinal fluid.

EXPERIMENTAL. A dog was placed under Nembutal anesthesia and, in experiments upon aqueous humor, aqueous humor was aspirated from the right eye by corneal puncture. A blood sample was withdrawn immediately after from the jugular or femoral vein, the withdrawal being

[1] This research was aided by a grant from the Abbott Laboratories.

followed by injection of sorbitol[2] in 50 per cent solution. After the lapse of a certain time, the aqueous humor was withdrawn from the left eye and a blood sample taken from the vein on the side opposite to that used for injection. Preliminary experiments showed reducing substances in the aqueous humors of the left and right intact eyes to be equal. It was also shown experimentally that after withdrawal of the aqueous humor from an eye, a second aqueous humor forms rapidly, which differs in composition from the original, bearing a closer resemblance to blood. Thus, to obtain a true estimate of the passage of sorbitol into the intact eye, the eye from which the first sample of aqueous humor was withdrawn could not be used as the source of the second aqueous sample. In experiments upon cerebrospinal fluid, the fluid was withdrawn by a suboccipital puncture with a hypodermic needle. Only amounts necessary for the experiments were taken. Glucose and sorbitol determinations were performed on all samples of blood, aqueous, or spinal fluid taken.

To determine sorbitol, a modification of the micromethod of Silberstein, Rappaport, and Reifer (5) was developed. These investigators precipitate the protein from 0.1 cc. of fluid (blood, serum etc.) by means of silicic acid.[3] The precipitate is washed and the filtrate plus washings heated in acid solution with periodate for 20 minutes. After addition of K_2HPO_4 and KI the excess periodate is titrated with thiosulfate. This method was modified by us in the following manner:

a. After the protein precipitation, the total volume in the reaction tube is made up to 8 cc.; the mixture is shaken, filtered through a dry filter, and a 4 cc. aliquot of the filtrate used for the periodate reduction. We feel that this procedure obviates the danger of loss of sorbitol through insufficient washing of protein, beside being more convenient.

b. The heating period was extended from 20 to 25 minutes. Experiments with sorbitol solutions showed this period of time to be more safely in the plateau of periodate reduction than the shorter period.

Periodate reduction is a function not only of the sorbitol in the filtrate but of glucose and other reducing substances as well. Consequently, glucose must be determined independently and subtracted from the periodate value. Benedict's method for glucose was used for this purpose (without bisulfite, so that other reducing substances would be included in this value). Silberstein, et al., give a factor by which glucose could be converted into cubic centimeters thiosulfate, and after subtraction of the thiosulfate equivalent of the glucose value, the factor could be used to calculate milligrams per cent of sorbitol. We were unable to verify the factor of these

[2] Courtesy of Abbott Laboratories.

[3] In the report of Silberstein et al. it is written that the silicic acid solution for protein precipitation is prepared from $Na_2SiO_3 \cdot 8H_2O$. This is probably a misprint, the salt being $Na_2SiO_3 \cdot 9H_2O$.

workers, finding, indeed, that addition of the same quantity of sorbitol or glucose to samples of blood from different dogs produced different amounts of reduction of periodate; that this was not a result of faulty technique is indicated by the fact that in samples of the same blood, added sorbitol gave the same factor on 4 successive days. To take into account

TABLE 1

Passage of sorbitol from blood into aqueous humor and cerebrospinal fluid

DOG NUMBER	WEIGHT OF DOG	50 PER CENT SORBITOL INJECTED	TIME AFTER SORBITOL INJECTION	SORBITOL IN AQUEOUS	SORBITOL IN CS FLUID	SORBITOL IN BLOOD	SORBITOL IN 2ND AQUEOUS	GLUCOSE IN BLOOD		GLUCOSE IN AQUEOUS		GLUCOSE IN CS FLUID		GLUCOSE IN 2ND AQUEOUS	
								Before sorbitol injection	After sorbitol injection	Before sorbitol injection	After sorbitol injection	Before sorbitol injection	After sorbitol injection	Before sorbitol injection	After sorbitol injection
	lbs.	*cc.*	*min.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
1	17	27	100	13		65		90	100	76	80				
2	20	26	70	14		58		90	95	81	90				
3	15	30	125	11		43		90	81	86	81				
4	17	28	95	7		51	116	87	84	84	94			84	91
5	16	28	40	0		183	175	87	119	85	92			85	105
6	35	52	90	25		46		94	114	84	97				
7	21	32	50	5	8	94		84	93	78	82	68	70		
8	16	30	85		28	40		91	93			90	93		
9	25	35	70		0	37		88	92			68	88		
10	18	32	65		2	50		88	69			68	55		
11	30	50	70		5	44		91	141			66	109		
12	22	35	100		5	17		73	98			64	64		
13	20	30	50		27	85		110	113			113	132		
14	18	28	45	25	4		81			74	104	63	82		
15	19	30	45	7	8		103			73	78	61	70	73	100
Average:															
Aqueous-blood.....				10		78									
Spinal fluid-blood					10	52									
Spinal fluid-aqueous ...				12	7										

the effect of each blood, aqueous or spinal fluid upon periodate reduction, known amounts of sorbitol and glucose were added to samples of the fluid being tested; a factor was thereby obtained for each.

RESULTS. In table 1 are given data on the passage of sorbitol from the blood into the aqueous humor (dogs 1–7) and into the cerebrospinal fluid

(dogs 7–13). The amounts of sorbitol appearing in the aqueous of the intact eye are compared with those passing into the spinal fluid in 3 cases (dogs 7, 14, 15), and with the quantities appearing in the reformed aqueous

TABLE 2

Passage of glucose from blood into aqueous humor and cerebrospinal fluid

DOG NUMBER	WEIGHT OF DOG	50 PER CENT GLUCOSE INJECTED	TIME AFTER INJECTION OF GLUCOSE	GLUCOSE IN AQUEOUS		GLUCOSE IN CS FLUID		GLUCOSE IN BLOOD		GLUCOSE IN 2ND AQUEOUS	
				Before glucose injection	After glucose injection	Before glucose injection	After glucose injection	Before glucose injection	After glucose injection	Before glucose injection	After glucose injection
	lbs.	*cc.*	*min.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
16	20	30	50	65	263	61	141	82	207	65	344
17	19	30	55	92	347	71	143	93	208	92	425
Average				78	305	66	142	87	207	78	384

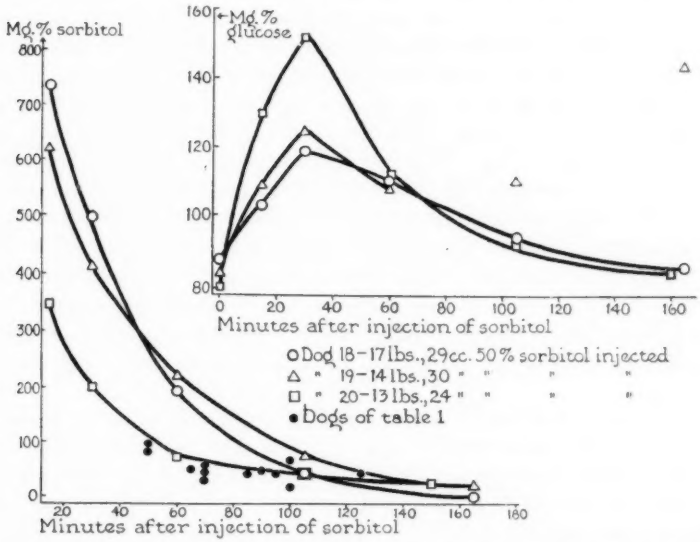

Fig. 1. The sorbitol and glucose content of the blood after intravenous injection of sorbitol.

(aqueous of same eye from which a sample was previously taken) in 4 cases (dogs 4, 5, 14, 15). The glucose levels for the aqueous, spinal fluid and blood before and after injection of sorbitol are also shown in table 1.

As a basis for comparison, glucose was injected intravenously into two

animals (dogs 16, 17), and passage into the aqueous humor and spinal fluid was measured. The results are shown in table 2.

The disappearance of sorbitol from the blood was followed in three animals (dogs 18–20), and the data plotted as a curve in graph 1. The corresponding glucose values of the blood were also measured, and their curve drawn on graph 1 as well. The points where the blood sorbitol values of table 1 fall are also shown in the graph.

Discussion. From the data given in table 1, it is evident that only a relatively small amount of sorbitol passes from the blood into the aqueous humor and cerebrospinal fluid. There is no apparent correlation between the amount found in these fluids and the amount injected or the time after the injection that the sample was withdrawn, the ratio varying with each animal. The averages shown in table 1, although of limited significance, since they cover variations in time and in sorbitol injected, indicate quite definitely that there does exist a barrier against passage of sorbitol into the spinal and ocular fluids. Thus, sorbitol satisfies the criterion of an osmotic agent used to reduce intracranial and intraocular pressures in not readily passing into the cerebrospinal fluid and aqueous humor. That the barrier is readily passed by glucose is indicated by the data in table 2. The increase in the glucose content of the aqueous and spinal fluid is quite marked when the sugar is injected into the blood stream.

As mentioned previously, after withdrawal of the aqueous humor, it is reformed in a short time. That an altered permeability of the chamber barrier occurs (disregarding theories for the mode of formation of the aqueous humor) under this circumstance is shown in the sorbitol and glucose values given for this reformed aqueous humor in tables 1 and 2. The fact that these values are higher in the reformed aqueous than in the aqueous of the untouched eye indicates an increased permeability. A gross increase in the protein content of the reformed aqueous over that of the original aqueous, as shown by protein precipitation, confirms this observation.

That sorbitol is eliminated quite rapidly from the blood is shown in graph 1. At the end of 165 minutes, dog 18 had 19 mgm. per cent sorbitol remaining in the blood, and dog 19 had none. Dog 20 had 25 mgm. per cent blood sorbitol at the end of 150 minutes. Thus, it may be said that in three hours very little sorbitol remains in the blood. That the blood sorbitol curve of dog 20 is probably the most nearly typical of the three is indicated by the fact that the values of blood sorbitol taken from table 1 fall closer to this curve than to the others.

The rise of blood glucose after injection of sorbitol, as shown in graph 1, is of interest. The blood glucose appears to reach a peak in about thirty minutes, after which it falls to approximately the original level. There is no evident explanation for the secondary rise in blood glucose of dog 19.

This glucose increase, as also noted by Myers (6), may be explained on the basis of conversion of sorbitol into glucose in the body. There exists a possibility, however, that the increase is a result of stimulation of liver glycogen breakdown. A glucose rise, although not so marked, has been noticed upon injection of sucrose as well (4). It is not of such magnitude, however, as to affect the virtue of sorbitol as a therapeutic agent for lowering high intraocular and intracranial tensions.

SUMMARY

1. A modification of the micromethod of Silberstein, Rappaport, and Reifer for sorbitol determination was developed.

2. It was shown that sorbitol injected into the blood stream passes into the aqueous humor and cerebrospinal fluid in relatively small amounts.

3. Sorbitol in the blood is rapidly eliminated, very little remaining at the end of three hours.

4. Sorbitol in the blood gives rise to a temporary increase in the blood glucose value.

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THE EFFECTS OF INSULIN AND GLYCINE ON HEPATIC GLUCOSE OUTPUT IN NORMAL, HYPOPHYSECTOMIZED, ADRENAL DENERVATED, AND ADRENALECTOMIZED DOGS

LATHAN A. CRANDALL, JR. AND IAN S. CHERRY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

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In her recent review of the relation of the pituitary gland to carbohydrate metabolism, Russell (1) has well summarized the problems connected with the changes produced by hypophysectomy. Extensive citations of the literature prior to 1937 will be avoided in the present report by assuming a knowledge of the material contained in this review. Russell has pointed out that many of the known effects of pituitary ablation may be explained by assuming that this gland either controls gluconeogenesis or exercises an influence on carbohydrate oxidation.

It was in the hope of throwing light on these and other incidental problems that we began a study of the effects of hypophysectomy by a direct method which consisted in analyzing the inflowing and outflowing blood of the liver for glucose, lactic acid, and urea in unanesthetized animals. Because the insulin sensitivity of hypophysectomized animals resembles that produced by adrenalectomy or adrenal denervation, animals so operated were also studied. On two previous occasions (2, 3) we have shown that anesthesia and acute surgical procedures may utterly distort the normal processes of carbohydrate metabolism. We have therefore, as previously, carried out our experiments without anesthesia on dogs angiostomized according to the technique of London (4). In general the methods employed were those used in previous studies on angiostomized animals (2, 3). We have now had dogs survive for more than two years with cannulae on the hepatic and portal veins. In some cases the cannulae will be pulled under the skin unless the animal is inspected regularly, but in many instances such preparations are as usable after having been put aside without attention for a year as they were when first made. The cannula may become filled with a hardened mass of detritus and secretion when not in regular use, but this is easily removed.

All determinations of "true" blood sugar and of blood lactic acid were performed in duplicate. Blood urea nitrogen was determined (in dupli-

cate) by the method of Folin and Wu (5); control determinations indicated that differences of 0.3 mgm. of nitrogen per 100 cc. of blood could be regarded as significant. Hypophysectomy was performed upon dogs that had been previously angiostomized. The pituitary gland was visualized by a temporal approach. It was dissected free of the sella and the stalk cut cleanly through by a small snare (nasal polyp type) passed up around the gland. Any remaining fragments that could be seen were dissected away with forceps. Examination of the gland showed that in most cases the entire anterior lobe and the greater part of the posterior could be cleanly removed by this method. The successful removal of the anterior lobe is attested by the fact that convulsions or coma were uniformly produced in our hypophysectomized animals by 15 units of insulin, whereas this amount of insulin has never caused any apparent symptoms in normal animals. When the animal was to be subjected to adrenal denervation, the right adrenal was completely removed at the time that the portal and hepatic veins were prepared for angiostomy by the attachment of small portions of omentum. At a second operation the cannulae were sewed to the veins and the left adrenal gland denervated by section of the greater and lesser splanchnic nerves and by sufficient dissection around the adrenal to permit lifting the upper pole of the gland from its bed. Completeness of adrenal denervation is also shown by the consistent shock produced in all our preparations by 15 units of insulin. Adrenalectomized animals were prepared by removal of the right adrenal gland at the time of the second operation for angiostomy, and removal of the left gland when the animals had fully recovered from previous surgery and the cannulae were functioning well.

Normal, hypophysectomized, adrenal denervated, and adrenalectomized dogs were studied with respect to retention of glucose and retention or output of lactic acid by the liver following the subcutaneous injection of 15 units of insulin. The same observations plus the hepatic output of urea after the administration of 20 grams of glycine by mouth were made on normal and hypophysectomized animals. Calculations of the effect of the liver on blood sugar, lactic acid, and urea were based upon nearly simultaneous samples of arterial, portal venous, and hepatic venous blood taken immediately before and 20 and 60 minutes after the administration of insulin, or immediately before and 30 and 90 minutes after the giving of glycine by stomach tube. Arterial blood was obtained from the femoral artery by direct puncture with a 23 gauge needle. The training of animals and other precautions to avoid excitement have been discussed previously (3).

The data obtained on the various groups of animals were subjected to statistical analysis by the usual methods for determination of the means,

standard errors of the means, and standard errors of the differences of the means.

Normal dogs. In tables 1 and 2 are given the effects of 15 units of insulin (subcutaneous) and 20 grams of glycine (oral), respectively, on the arterial blood sugar level and the output of glucose by the liver per 100 cc. of blood. Individual lactic acid values are not shown because the changes produced, if any, were too slight and irregular to be statistically significant. We have shown previously that on the average there is neither

TABLE 1

Effect of insulin on hepatic glucose output and blood sugar level in normal dogs

Samples taken immediately before, and 20 and 60 minutes after, the subcutaneous injection of 15 units of insulin. Dogs divided into two groups; group A—control output less than 10 mgm. glucose per 100 cc. of blood; group B—control output greater than 10 mgm. per 100 cc.

	DOG. NO.	ARTERIAL SUGAR LEVEL			HEPATIC GLUCOSE OUTPUT		
		C	20 min.	60 min.	C	20 min.	60 min.
Group A	2	78	66	34	4	6	9
	14	67	45	37	9	12	16
	7	81	65	32	9	3	10
	10	62	43	28	8	14	10
	3	69	50	41	5	11	10
	5	77	62	54	5	7	10
	4	90	84	39	8	9	11
	6	69	56	51	2	7	10
Means of group A.		74.1 ±3.2	58.9 ±4.6	39.5 ±3.2	6.2 ±0.9	8.6 ±1.3	10.7 ±0.8
Group B	11	80	75	32	12	10	12
	8	79	52	26	31	5	24
	9	94	69	44	31	5	28
	6	78	51	52	16	10	11
Means of group B.		82.8 ±3.7	61.8 ±6.0	38.5 ±6.2	22.5 ±5.0	7.5 ±1.4	18.7 ±4.3
Means of all animals		77.0 ±2.6	59.8 ±3.6	39.2 ±2.7	11.7 ±2.8	8.3 ±0.9	13.4 ±1.8

retention nor output of lactic acid by the liver in the normal fasting animal (3). Insulin produced no change in the blood lactic acid level in 9 of our 12 animals; there was a slight increase in blood lactic acid in 2 and a slight decrease in 1. The effect of the liver upon the lactic acid content of the blood was unchanged after insulin in 6 cases; there was a tendency toward increased retention in 4 instances and toward increased output in 2. There were no observable effects of insulin upon retention of glucose or output of lactic acid by the intestinal tract as judged by arterio-portal differences.

We have shown previously on the basis of data from 47 dogs that the average output of glucose by the liver is 9.1 mgm. per 100 cc. of blood. Inspection of the data in table 1 seems to indicate that the response of the liver to insulin varies according to whether it is adding more or less than the average amount of glucose to the blood stream. To illustrate this difference the animals in table 1 have been divided into two groups as follows: group A—average or less than average glucose output in the control period; group B—a control output above average. The correlation coefficients have been calculated for the relationship between the response to insulin, as determined by the changes in output at the 20 and 60 minute post-insulin periods, and the control level of glucose output. The correlation between control (pre-insulin) output and change in output at 20 minutes (difference between control and 20 minute output) has a co-

TABLE 2

Effect of glycine on hepatic glucose output and arterial glucose level in normal dogs

Samples taken immediately before, and 30 and 90 minutes after, 20 grams glycine by stomach tube (one experiment 4 grams glycine intravenously).

DOG NO.	ARTERIAL GLUCOSE LEVEL			HEPATIC GLUCOSE OUTPUT			HEPATIC UREA OUTPUT		
	C	30 min.	90 min.	C	30 min.	90 min.	C	30 min.	90 min.
13	77	89	78	9	27	11	0.3	−0.1	2.1
7	71	98	81	4	14	10	0.5	0.4	2.1
11	81	86	88	12	14	13	0.7	1.4	2.9
10	65	76	64	17	24	21	1.8	2.1	3.2
9	84	95	96	17	23	21	−0.4	1.6	4.1
8	64	80	82	6	19	16	−0.2	0.6	2.8
Means . . .	73.7 ±3.4	87.3 ±3.5	81.5 ±4.4	10.8 ±2.2	20.2 ±2.2	15.3 ±2.0	0.45 ±0.25	1.0 ±0.32	2.87 ±0.31
24*	84	102	87	23	32	21			

* Four grams glycine intravenously; samples taken 15 and 45 minutes after injection.

efficient (r) of -0.96; r for the 60 minute period is -0.85. Correlation coefficients for such small groups are subject to well known errors, but as determined by table V. A. of Fisher (6) these values for r are significant since the probability of their correctness is greater than 99 in 100. We may therefore conclude that the higher the fasting glucose output of the liver in terms of milligrams per 100 cc., the more likely it is that insulin will decrease the output, whereas, if the fasting output is low insulin will cause an increase. If we assume that the glucose liberated by the liver is derived from the glycogen store of that organ, we may further conclude that insulin may either increase or depress glycogenolysis depending upon the rate of glycogenolysis at the time the insulin is injected. This last statement requires the further assumption that the blood flow through the liver is constant. It would obviously have been desirable to determine

blood flow in the portal vein and hepatic artery, but technical difficulties kept us from attempting to make such determinations at the time of drawing blood samples in these angiostomized animals. The allowances that must be made for variations in hepatic blood flow will be considered below.

Inspection of table 1 also suggests a correlation between the fasting blood sugar level and fasting hepatic glucose output. Accordingly, the correlation coefficient for this possible relationship has been determined. For the 12 cases in table 1, $r = 0.45$ and does not indicate a significant relationship. This value for r approaches significance closely enough, however, so that we have calculated the same coefficient for a larger series. When an additional 12 unselected and consecutive observations on fasting arterial blood sugar level and fasting hepatic glucose output taken from other experiments are added to the data in table 1, r for the enlarged series becomes 0.54 and the probability that this value would occur by chance is less than 1 in 100. From the enlarged series, therefore, we may conclude that when the fasting glucose output of the liver is high the blood sugar is also likely to be above the average and *vice versa*. The values of r for the relationship between fasting blood sugar level and the change in output that occurs after insulin are -0.51 for the 20 minute and -0.42 for the 60 minute period. These figures do not prove a relationship, but might become significant if the series were extended. Since we have observed a significant correlation between the control glucose output and the effect of insulin, and between the fasting blood sugar level and the control glucose output, it would seem that all three might be shown to be interrelated if a sufficiently large number of animals were observed.

The mean arterial blood sugar level exhibits the usual and expected decrease after insulin. The differences of the means of the control, 20 and 60 minute blood sugar values are greater than twice the standard errors of these differences in both group A and group B as well as for the entire series; all the observed changes in mean blood sugar level are therefore significant.

In group A (low fasting hepatic output) the difference between the mean control output and the mean output 20 minutes after insulin is 2.6 ± 1.6; this difference is therefore not statistically significant. At 60 minutes, however, the mean output is 4.5 mgm. greater than the fasting value and the S. E. of the difference is 1.2; the difference is highly significant.

In the high output group the difference between control and 20 minute mean outputs is 15.0 ± 5.54, between control and 60 minute is 3.8 ± 6.69, and between 20 and 60 minutes is 11.2 ± 4.73. In this group, therefore, there is a significant decrease in hepatic glucose output in the first 20 minutes followed by a significant rise at 60 minutes to an output not significantly different from that present before administration of insulin.

The homeostatic mechanism by which a low blood sugar level after insulin is related to an increased hepatic glucose output is clearly illustrated by our data. These observations are in complete accordance with the generally accepted interpretations of insulin action. It is interesting that by such direct observations one is able to show inhibition of hepatic glucose output and presumably inhibition of glycogenolysis, since it is well known that this effect of insulin is difficult to demonstrate by other methods. It may be pointed out that the blood sugar level at which the homeostatic mechanism (epinephrine secretion) for the restoration of the blood sugar level comes into play is below 60 mgm. per 100 cc. From inspection of the figures for individual animals one is inclined to place the level at which medullo-adrenal secretion is stimulated at a point between 50 and 60 mgm. per 100 cc.

The effects on hepatic glucose and urea outputs of the administration of glycine (20 grams by mouth) are shown in table 2. These observations serve as controls for the similar experiments on hypophysectomized animals that are reported below. In themselves, they are indicative of the rapidity with which the liver is capable of dealing with this simple amino acid. It will be seen that the mean glucose output of the liver has doubled within 30 minutes after the administration of glycine. It is interesting to recall that Wilson and Lewis (7) could show no glycogen formation when glycine was given to rats by stomach tube, although in our animals glucose formation is demonstrated both by the increased glucose output from the liver and by the increase in blood sugar level.

If one assumes that both carbon atoms of the glycine molecule are used for the construction of glucose, then an increase in urea nitrogen output of 1 mgm. would be equivalent to 4.7 mgm. of glucose. The increases in urea nitrogen output observed at the 90 minute interval would account for 9 to 19 mgm. of glucose. Although an average increase of 9.4 mgm. of glucose output was observed at the 30 minute period, the increase has fallen to 4.5 mgm. at 90 minutes. The lag of urea liberation by the liver in comparison to the liberation of glucose is worthy of note. Presumably amino acid is deaminized prior to the formation of glucose from the remainder of the molecule; one is, therefore, led to suspect that assembling the urea molecule is a slow process or that urea is retained in the liver after formation.

The blood lactic acid level, the effect of the liver on the blood lactic acid, and the arterio-portal glucose and lactic acid differences appeared to be unaffected by the administration of glycine.

Hypophysectomized animals. The same procedures that were discussed above for normal dogs were applied to hypophysectomized animals. Table 3 shows the effects of insulin administration on arterial blood sugar and hepatic glucose output in 11 experiments on 7 dogs. Before discussing

the action of insulin in these animals, it should be pointed out that the fasting blood sugar level is below that of the normal group, and the mean hepatic glucose output is 4.0 mgm. per 100 cc. as compared with a mean of 11.7 mgm. for the normals. In fact, in only two of these dogs does the output approach the mean value of 9.1 mgm. for normal dogs as previously determined on a large series (3). It has been shown by Cherry and Crandall (8) that the average rate of blood flow through the liver in hypophysectomized animals is not significantly different from the normal. We may, therefore, assume that the amount of glucose supplied to the body by the liver is actually somewhat less than half as great in the hypo-

TABLE 3

Effect of insulin on glucose output by the liver in hypophysectomized dogs

Samples taken immediately before and 20 and 60 minutes after 15 units insulin subcutaneously.

DOG NO.	ARTERIAL SUGAR LEVEL			HEPATIC GLUCOSE OUTPUT			RESULTS
	C	20′	60′	C	20′	60′	
H4	82	57	47	8	5	4	Shock 50 minutes, convulsions 2 hours
H14	66	44	25	9	4	5	Shock 90 minutes
H3	75	70	34	4	3	3	Mild shock 90 minutes
H6A	68	44	28	4	1	3	Convulsions 60 minutes
H6A	72	45	42	3	0	0	Shock 60 minutes
H6A	70	33	19	5	1	4	Shock 60 minutes
H6	58	45	26	0	1	−1	Severe shock 60 minutes
H5	68	49	30	5	6	6	Shock 60 minutes
H5	65	44	34	4	2	5	Shock 60 minutes, convulsions 2 hours
H4	61	44	35	2	4	4	Shock 90 minutes
H6	62	63	26	0	−1	1	Shock 2 hours
Mean ..	67.9 ±2.1	48.9 ±3.1	31.5 ±2.4	4.0 ±0.8	2.4 ±0.7	3.1 ±0.7	

physectomized dog as in the normal. This would account for the tendency toward a starvation hypoglycemia in these animals.

The differences between the mean arterial blood sugar levels of the hypophysectomized and normal animals, and the standard errors of the differences, at the control, 20 and 60 minute periods are: 9.1 ± 3.2, 11.1 ± 4.6, and 7.7 ± 3.7. Since the differences are in each case greater than twice the standard error, they may be considered significant. It is apparent that the rate of fall in blood sugar is no greater in the hypophysectomized dog than in the normal. Soskin (9) has presented evidence indicating that a low blood sugar level is accompanied by a decreased ability of the tissues to utilize glucose. Decreased hepatic output and decreased utilization appear to balance in such a manner that the rate of decrease in blood sugar after insulin is parallel to that in the normal, although the actual blood sugar level is consistently and significantly lower.

The mean glucose output by the liver shows a slight decrease after insulin. The change is not statistically significant, since the standard error of the difference between the mean control and mean 20 minute outputs is ±1.1 and the difference itself is 1.6. The decrease in output after insulin is most marked in those animals exhibiting the highest control output, and this trend may be significant since it is seen again in both the adrenalectomized and adrenal denervated dogs. The hepatic glucose output of hypophysectomized dogs should presumably be compared with group A of the normals, since the former animals in every case have a control output below 10 mgm. per 100 cc. When this is done the differences between mean outputs and the standard errors of the differences are found to be as follows: control 2.2 ± 1.2, 20 minutes 6.2 ± 1.4, and 60 minutes 7.6 ± 1.0. The difference in the control outputs is within the limits of error, while the differences after insulin are definitely significant. If the same comparison is made between the values for hypophysectomized dogs and the mean outputs for the entire group of normal animals the values are: 7.7 ± 3.0, 5.9 ± 1.1, 10.3 ± 1.9. The differences are significant in each case and there is a suggestive increase at the 60 minute period.

It seems permissible to conclude that the response of the liver to insulin hypoglycemia is altered by hypophysectomy. The change that occurs is a failure of the homeostatic mechanism which, in the normal animal, increases the hepatic glucose output at levels of blood sugar below 60 mgm. per cent. In the hypophysectomized animal, on the contrary, any change in glucose output appears to be in the direction of a decrease.

In confirmation of many previous observers we have found (as noted in table 3) that the hypophysectomized dog exhibits severe shock after an injection of insulin (15 units in our experiments) which produces no apparent symptoms in normal animals. We allowed our animals to develop coma or convulsions as an indication of anterior lobe deficiency, but treatment with intravenous glucose was given as a life saving measure as soon as severe symptoms developed. It was often necessary to repeat intravenous glucose injections over several hours to prevent recurring coma or convulsions.

Determinations of lactic acid in portal and hepatic venous and in arterial blood again failed to reveal sufficiently consistent changes to warrant tabulation of the results. The arterial lactic acid level remained unchanged in 3 animals, increased somewhat in 6, and decreased in 2. Insulin appeared to have no effect on the action of the liver on blood lactate in 6 cases, while there was increased retention after insulin in 5. The arterio-portal glucose differences were unaffected by insulin.

Our experiments with the oral administration of glycine to hypophysectomized dogs were unsatisfactory, since these animals tended to vomit a

small part of the solution a half-hour to an hour after its administration. The amount vomited was judged to be too small a fraction to be particularly significant, but we considered the vomiting as suggestive of delayed gastric emptying. In this respect the results with glycine in the hypophysectomized animals cannot be considered comparable to those in normal dogs and our series was intentionally limited. One fact, however, is clearly demonstrated by the data in table 4. The hypophysectomized animal is perfectly capable of prompt conversion of this amino acid to glucose. The increases in arterial blood sugar and in hepatic glucose output are as prompt and marked as in the normal animal. There is also an increased hepatic output of urea in the 3 animals studied. We may conclude that failure of the liver to maintain the blood sugar level in the

TABLE 4

The effect of 20 grams of glycine by mouth in hypophysectomized dogs

Hepatic venous, portal venous, and arterial blood samples taken immediately before, and 30 and 90 minutes after, administration of glycine.

DOG NO.	ARTERIAL GLUCOSE LEVEL			HEPATIC GLUCOSE OUTPUT			HEPATIC UREA OUTPUT		
	C	30′	90′	C	30′	90′	C	30′	90′
H3	83	85	74	4	19	14	0.2	0.0	0.9
H3	65	81	72	7	17	15	0.5	0.6	1.6
H7	70	96	78	4	9	7			
H7	74	80	81	4	24	28	1.1	2.4	0.5
Average....	73.0	85.5	76.7	4.7	17.2	16.0	0.6	1.0	1.0
H3*	70	85	75	6	21	14			

* Four grams glycine intravenously; samples taken 15 and 45 minutes after injection.

hypophysectomized dog is not secondary to an inability to utilize an amino acid for this purpose when the amino acid is present in excess.

No significant changes in lactic acid metabolism were observed after the administraton of glycine to hypophysectomized dogs.

Adrenal denervated dogs. These animals resembled the hypophysectomized preparations in the symptoms that appeared within 1 hour after insulin. If anything, coma or convulsions appeared earlier and were more severe in the adrenal denervated dogs than in those that were hypophysectomized. It is our impression, however, that recovery is not as long delayed as after hypophysectomy. This impression is based on the amount of treatment required. In both types of animals the symptoms produced by 15 units of insulin were so striking and regular in appearance that they were made the subject of repeated class demonstrations.

The effect of insulin on arterial sugar level and hepatic glucose output in the adrenal denervated dogs is shown by the data in table 5. The means of the control values for the adrenalectomized dogs are not significantly lower than the means for normal animals, the differences with their standard errors being 4.9 ± 4.5 for control arterial blood sugar and 5.2 ± 2.9 for hepatic output. When compared with group A (those with low control hepatic output) of normal animals the differences are still less, being 2.0 ± 4.5 for blood sugar and 0.3 ± 1.0 for hepatic output. This suggests that in those animals with a high fasting blood sugar level and hepatic output (group B of table 1), the liver is being influenced by a secretion of epinephrine. In fact, from examination of the control hepatic glucose outputs of the normal, hypophysectomized, adrenal denervated,

TABLE 5

The effect of 15 units of insulin subcutaneously on arterial blood sugar level and hepatic glucose output in adrenal denervated dogs

Samples taken immediately before, and 20 and 60 minutes after, insulin.

DOG NO.	ARTERIAL GLUCOSE LEVEL			HEPATIC GLUCOSE OUTPUT			RESULTS
	C	20′	60′	C	20′	60′	
Ad2	72	24	14	8	11	10	Convulsions 45 minutes
Ad3	65	53	25	12	4	5	Shock 45 minutes, convulsions 60 minutes
Ad4	67	50	19	7	4	4	Shock 50 minutes, convulsions 90 minutes
Ad1	67	60	30	3	2	1	Shock 60 minutes
Ad3	80	45	30	2	3	2	Shock 90 minutes
Ad4	91	54	25	0	7	5	Coma 60 minutes
Ad2	75	27	21	12	8	7	Shock 30 minutes, convulsions 60 minutes
Ad5	60	50	20	8	7	5	Severe shock 60 minutes
Mean ..	72.1 ±3.5	45.4 ±4.6	23.0 ±2.0	6.5 ±1.6	5.8 ±1.1	4.9 ±1.0	

and adrenalectomized (see below) animals, one is tempted to hypothesize that whenever the output in the fasting state exceeds the normal average by an appreciable amount epinephrine is being secreted.

Although the control values in the adrenal denervated animals are not significantly different from those of the normal group, the fall in blood sugar after insulin is more precipitate and there is no increase in the hepatic glucose output (the slight decrease in mean hepatic output is not statistically significant). The differences of the means of arterial blood sugar for the adrenal denervated and low normal groups after insulin are 13.5 ± 6.7 (20 min.) and 16.5 ± 3.8 (60 min.). For hepatic output they are 2.8 ± 1.6 (20 min.) and 6.1 ± 1.4 (60 min.). Differences of similar significance are obtained when comparison is made with the means for all normal values. These findings are similar to those obtained with the

hypophysectomized animals, except that in the presence of adrenal denervation the fall in blood sugar is slightly more precipitate.

Lactic acid determinations in these experiments revealed no significant departure from the normal.

Adrenalectomized animals. In 3 animals the second adrenal was removed after the portal and hepatic cannulae had become securely fixed. These dogs were maintained on a low potassium high sodium diet as described by Allers and Kendall (10), with occasional injections of cortical extract at critical periods.[1] Only blood sugar was determined in these experiments, since analyses for lactic acid require the removal of larger amounts of blood than such dogs tolerate. The effects of insulin are shown by table 6. The decreases in blood sugar are in the same range as those found in adrenal denervated animals, and there is the same tendency toward a decrease in hepatic glucose output that was observed in both the

TABLE 6

Effect of 15 units of insulin on adrenalectomized dogs maintained on a low potassium high sodium diet

DOG NO.	ARTERIAL GLUCOSE LEVEL			HEPATIC GLUCOSE OUTPUT			RESULTS
	C	20′	60′	C	20′	60′	
A3	73	63	18	16	11	7	Severe convulsions 60 minutes
A3	75	30	22	3	2	0	Coma 60 minutes
A2	63	44	14	9	7	2	Convulsions 50 minutes
A4	73	55	28	2	1	2	Severe shock 55 minutes
Mean...	71.0	48.0	20.5	7.5	5.2	2.7	

hypophysectomized and adrenal denervated preparations. Coma or convulsions appeared regularly within about one hour after 15 units of insulin.

Critique of angiostomy method. The angiostomy method has been criticized on the ground that changes in concentration of substances in the blood passing through an organ may not not be indicative of retention or output per unit time. Soskin *et al.* (11) have even implied that in the absence of blood flow determinations the method can indicate nothing more than the direction of movement into or out of the liver. However, a recalculation of their data assuming a constant blood flow of 27 cc. per kilo per minute as given for the liver by Blalock and Mason (12) leads us to believe that the omission of blood flow determinations would not have altered their conclusions.

[1] We wish to express our appreciation of the kindness of Dr. W. D. Allers who was responsible for the post-operative care of these animals.

Angiostomy permits the use of unanesthetized animals and eliminates trauma. The qualitative differences between observations on carbohydrate metabolism performed under acute conditions (13, 14, 15) and by the angiostomy method (2, 3, 4, 16) indicate that derangements produced by anesthesia and surgical trauma may lead to complete reversals of hepatic activity. We have therefore omitted measurements of blood flow because at the present time they appear to be impractical except in acute experiments.

Interpretations of data from angiostomy experiments where blood flow is unknown must be made in the light of the following question: *what must be the magnitude of variations in intake or output of a substance per unit volume of blood before it can be assumed that they indicate changes in output per unit time?* We have reviewed the determinations of hepatic blood flow reported in the literature (12, 17, 18, 19, 20). All are in general agreement, although in only one instance (12) were the studies made on unanesthetized animals. Blood flow variations in individual dogs are surprisingly small. Greater differences occur when various animals are compared. We have analyzed the data by Blalock and Mason (12), since they did not use anesthesia and also report the largest number of experiments. The mean blood flow for the 13 dogs reported by them is 28.6 cc. per kilo per minute; the standard error of the mean is 1.83 and the standard deviation 6.3. On this basis a single observation would depart more than 44 per cent from the mean only once in twenty times. And if the mean hepatic retentions or outputs, in two series of 13 animals each, should differ by 13 per cent the difference would not be likely to be caused by variations in blood flow.

It should also be pointed out that statistical analysis of data, as in this communication, makes allowances for random blood flow variations as well as other random errors. The only systematic errors that might occur, as far as we are aware, are those produced by the administration of drugs or the experimental procedures involved. We know of no evidence that insulin produces a change in blood flow. If one were to assume that the results given in table 1 were secondary to circulatory changes produced by insulin it would be necessary to believe that in some normal animals insulin decreases hepatic flow while in others it produces first an increase and then a decrease. And to explain our data on the operated dogs it would be necessary to assume that adrenal denervation and hypophysectomy increase flow. But we have shown previously (8) that the circulation time through the liver is not affected by these surgical procedures. On the basis of present knowledge it seems reasonable to believe that the statistically significant differences reported here represent actual changes in hepatic output per unit time.

We do not wish to minimize the importance of variations in blood flow.

It is unfortunate that a number of investigators who have applied the angiostomy method have not taken blood flow variations sufficiently into account. We believe that conclusions from such studies should only be drawn when: 1, the number of experiments is sufficiently large to permit statistical analysis and thus rule out random errors due to irregularity of blood flow and other causes; 2, the effect of any procedures which may be suspected of causing systematic blood flow changes has been determined.

General discussion. The effect of insulin on hepatic glucose output in the normal animal, as found in our experiments, is in line with previous indirect evidence. Although the more common effect is to increase the liberation of glucose, it is especially interesting that in some animals with an output already high it is possible to demonstrate a temporary reduction. This is in accordance with the view that the primary effect of insulin is to promote glycogen storage and inhibit glycogenolysis and gluconeogensis. In the normal fasting dog this phase is evidently transient and promptly gives way to an increased output brought about by the response of the homeostatic mechanisms (epinephrine) to hypoglycemia.

Our findings offer an explanation of the hypersensitivity to insulin and the low fasting blood sugar of hypophysectomized animals. They are unable to respond to the hypoglycemic state by an increased liberation of glucose from the liver, which is already well below normal in its glucose output. The resemblance between the hypophysectomized and adrenal denervated or adrenalectomized dog in its response to insulin is striking, and lends attraction to the theory that the secretion of epinephrine is either diminished or that this hormone is rendered ineffective after pituitary removal. Both of these possible changes in the epinephrine mechanism have been denied by various investigators. If one accepts the evidence that epinephrine is secreted and is present in the adrenal gland in apparently normal amounts after hypophysectomy (21, 22) and that intravenous or intraperitoneal epinephrine has its usual hyperglycemic effect in the hypophysectomized animal (23, 24, 25), it becomes extremely difficult to explain the observed facts. In view of the difficulties that attend epinephrine assay and the good agreement between those who report a normal effect from this substance given intravenously or intraperitoneally, a study of the blood sugar level at which the adrenal medulla increases its secretion after insulin would seem in order.

If, as we have observed, the hepatic glucose output of fasting hypophysectomized dogs is in the neighborhood of 50 per cent of that in normal animals, an excessive glucose consumption by muscle and other tissues seems improbable. Any consumption over and above the amount of glucose liberated by the liver would necessarily be at the expense of muscle glycogen and the glucose of the body fluids. That an abnormally high

rate of glucose utilization and a reduced rate of liberation from the liver could coexist without a more precipitate drop in blood sugar level on fasting than occurs even in hypophysectomized animals appears unlikely.

Because an inability to carry out the process of gluconeogenesis, especially from amino acids, has been frequently suggested as at least a partial explanation of the changes occurring after hypophysectomy, we administered glycine and determined the output of glucose and urea from the liver. The results show that when an excess of glycine is available the liver is essentially normal in behavior even though anterior lobe deficiency and sensitivity to insulin are present. This does not support the view of Long (27) that there is a failure of gluconeogenesis from amino acids after hypophysectomy. Nor can it be taken as directly contradictory of Long's hypothesis, for the reaction to an overabundance of amino acid may be quite different from the ability to carry out gluconeogenesis from amino acids when their level in the blood is normal. One can only say that failure of gluconeogenesis from glycine could not be demonstrated by the methods employed.

It is evident that the present status of our knowledge does not permit the presentation of any complete hypothesis concerning the alterations in carbohydrate metabolism that occur after hypophysectomy. The demonstration of a decreased hepatic glucose output and failure of response to insulin in hypophysectomized dogs and adrenalectomized animals should aid in the later formulation of such a hypothesis.

SUMMARY AND CONCLUSIONS

Direct observations of the effects of insulin (subcutaneous) and glycine (oral) on glucose and lactic acid, in portal, hepatic, and arterial blood were made in normal, hypophysectomized, adrenal denervated, and adrenalectomized animals by the angiostomy technique without anesthesia. Retention or output by the liver is expressed in terms of milligrams of substance per 100 cc. of blood. A critique of the angiostomy method is presented in an effort to determine the degree to which our results may be regarded as quantitative. The data obtained have been subjected to statistical analysis. The observations found to be significant are as follows:

1. In normal dogs there is a positive correlation between fasting arterial blood sugar level and hepatic glucose output ($r = 0.54$ in 24 dogs). There is also a correlation showing an inverse relationship between fasting hepatic glucose output and the change in glucose output by the liver 20 minutes after insulin ($r = -0.95$ in 12 dogs). If glucose liberation by the liver in the fasting animal is assumed to represent the rate of glycogenolysis, this indicates that when blood sugar and fasting hepatic glucose output are high, insulin tends to suppress glycogenolysis (at least until

hypoglycemia occurs); if blood sugar and hepatic output are low there is no suppression of glycogenolysis and the liver reacts to the subsequent hypoglycemia by an increase in glycogenolysis.

2. Normal dogs react to the oral administration of 20 grams of glycine by an average increase of 17 mgm. in blood sugar and approximately 100 per cent increase in hepatic glucose output (30 min.). An increased hepatic output of urea lags behind the increase in glucose output.

3. Hypophysectomized dogs uniformly exhibit severe insulin shock about 1 hour after 15 units subcutaneously. The arterial blood sugar of such animals in the fasting state is lower than that of normal dogs and is lower than but parallel to the normal after the injection of insulin.

4. The fasting hepatic glucose output in hypophysectomized animals averages less than 50 per cent of the value for normal dogs, and does not increase during the hypoglycemic stage of insulin action as it does in normal animals.

5. The changes in hepatic glucose output and blood sugar level after glycine is administered to the hypophysectomized dog are comparable to those of the normal animal.

6. The fasting arterial blood sugar of adrenal denervated animals is within normal limits, but the fall in blood sugar after insulin is more abrupt and the level at one hour is definitely lower than normal. The fasting hepatic glucose output of the adrenal denervated dog is suggestively but not significantly lower than in the normal. As in the hypophysectomized preparation, insulin does not increase hepatic glucose output. These animals uniformly show insulin shock about 1 hour after injection.

7. Adrenalectomized dogs maintained on a high sodium low potassium diet exhibit blood sugar and hepatic glucose output values comparable to those of adrenal denervated dogs, and the changes after insulin injection are similar.

We have interpreted these results as showing that the liver is fundamentally concerned in the alterations of carbohydrate metabolism observed after hypophysectomy. Changes in the metabolism of other tissues are not excluded. Hypophysectomized animals show a lack of hepatic response to insulin that is similar to the condition present when the adrenal medullary mechanism has been eliminated.

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SENSITIZATION OF THE SUBMAXILLARY GLAND BY SYMPATHETIC DENERVATION

F. A. SIMEONE[1] AND J. P. MAES[2]

From the Department of Physiology in the Harvard Medical School

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Increased responsiveness after denervation has been demonstrated for a number of neuro-effector systems (cf. Cannon and Rosenblueth, 1937; Simeone, 1938a). Reference has been made to the denervated submaxillary gland as an organ peculiarly sensitive to the action of adrenaline (Florovsky, 1917; Sharpey-Schafer, 1924). The experiments upon which the statement is based, however, were done with acutely denervated glands. In fact, the only available data comparing the responses of the normal with those of the contralateral gland after long-standing sympathetic denervation are those of Fröhlich and Loewi (1910). In the one experiment reported, they were unable to detect any difference in the sensitivity to adrenaline on the two sides.

Maes (1938) has recently reported an increase in the sensitivity of the lachrymal gland to adrenaline, pilocarpine and acetylcholine 11 days or more after sympathetic denervation. Like the lachrymal gland, the submaxillary produces an external secretion, and one might expect sympathetic denervation to have similar effects on the two organs. In the absence of adequate data bearing on this supposition, it was thought of interest to test the effect of chronic sympathetic denervation on the responses of the submaxillary gland in the cat.

METHOD. Ten adult cats were used. The right or left submaxillary gland was denervated by excision of the superior cervical ganglion. The parasympathetic innervation was left intact. The experiments were done 47 to 90 days after denervation. Under urethane anesthesia (1.25 mgm. per kgm. intravenously) both adrenals were ligated, and the submaxillary ducts were exposed in the floor of the mouth through a ventral midline incision from the neck to the symphysis of the mandible. The hypoglossal nerves were excised. The lingual nerves were ligated and cut as close as possible to the mandible. They were cut again beyond the origin of the *chorda tympani* and formed convenient trunks for electric stimulation of that nerve. The superior cervical ganglion was excised or decentralized acutely on the intact side.

[1] Medical Fellow of the National Research Council.

[2] Fellow of the C. R. B. Educational Foundation.

The submaxillary ducts were distended with saliva by stimulation of the parasympathetic fibers in the isolated lingual nerve trunks and then cannulated with capillary glass pipettes. These were cut sufficiently short to lie in the wound along the natural course of the ducts without kinking. Each was connected through small flexible rubber tubing (internal diameter = 1 mm.) and a glass adapter to a second pipette. This, held vertically downward, ended in a capillary which caused the formation of very small drops. The end was oiled to make the drops fall more readily. The system was filled with salt solution to expel air and its patency was tested by observing the salivary flow after stimulation of the *chorda tympani*. The responses of the glands were recorded on a kymograph by means of signal magnets activated through a hand key as the drops fell from the pipettes. The same two pipettes were used for all the experiments interchangeably. On calibration, the volume of 100 drops was found to be 1.1 cc. for one and 1.0 cc. for the other. The drugs studied were adrenaline, acetylcholine, pilocarpine and cocaine. All injections were made into the saphenous vein.

During the course of other experiments, the secretion from the normal right and left submaxillary glands in response to adrenaline was tested in 8 cats by means of the above technique.

RESULTS. A. *Comparability of responses in right and left submaxillary glands of the normal cat.* In 6 of 8 cats tested, the responses of the right and left submaxillary glands to adrenaline were quantitatively alike. In the 2 remaining animals, the responses of the right gland were greater than those of the left but not significantly (less than 10 per cent). On the other hand, a striking variability was found in the sensitiveness of the glands in different animals. In the most sensitive, both submaxillary glands responded to as little as 5 γ of adrenaline injected intravenously. In the least sensitive, no response was obtained with less than 100 γ.

B. *Responses of normal and denervated submaxillary glands to adrenaline.* Ten animals were tested. In 6, the responses of the chronically denervated were more than 50 per cent greater than those of the contralateral, acutely denervated, control glands. In one case, the response of the denervated organ was 9 times that of the normal. In 2 of the 10 cats, the responses of the denervated side were about 20 per cent greater than those of the normal. In 1 of these, however, after the first few responses the secretion from the denervated side became 50 to 75 per cent greater than that from the normal side. In the remaining 2 of the 10 animals, no difference could be detected in the responses of the two sides. Figure 1 and table 1 illustrate typical instances.

C. *Responses of normal and denervated submaxillary glands to acetylcholine.* The sensitivity of normal and chronically denervated glands to acetylcholine was tested in 10 animals. In 3, the secretion from the de-

nervated was more than 50 per cent greater than that from the normal control gland. In 4 others, the responses were greater on the denervated

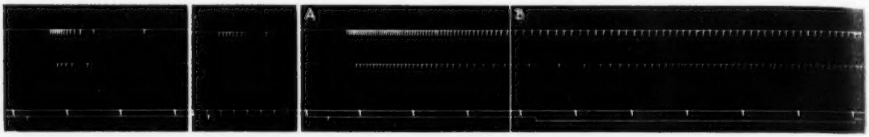

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Simultaneous record of drops of saliva from chronically denervated submaxillary gland (upper record) and acutely denervated gland (lower record). Urethane (1.25 grams per kgm. intravenously). Adrenaline (10γ) injected intravenously at signal. Time: 5 sec. Responses to acetylcholine in this animal were identical on the two sides.

Fig. 2. Simultaneous record of drops of saliva from chronically denervated submaxillary gland (upper record) and acutely denervated gland (lower record). Urethane (1.25 grams per kgm. intravenously). Acetylcholine (1.2 mgm.) injected intravenously at signal. Time: 5 sec.

Fig. 3. Same preparation as in figure 1. A. Pilocarpine (0.1 mgm.) injected intravenously at signal. B. 15 sec. later; 16 mgm. cocaine injected intravenously during signal.

TABLE 1

Responses of the chronically denervated and the normal submaxillary gland of a cat to increasing doses of adrenaline

DOSES OF ADRENALINE	RESPONSES (IN DROPS OF SALIVA)	
	Left gland (chronically denervated)	Right gland (acutely denervated)
γ		
25	3	0
50	7	0
100	24	11

TABLE 2

Responses of the chronically denervated and the normal submaxillary gland of a cat to increasing doses of acetylcholine

DOSE OF ACETYLCHOLINE	RESPONSES (IN DROPS OF SALIVA)	
	Left gland (chronically denervated)	Right gland (acutely denervated)
mgm.		
0.10	4	0
0.25	13	5
0.50	11	4

side, but only 15 to 30 per cent greater. In 3 of the 10 cats, the responses of the two sides were equal. Figure 2 and table 2 represent typical instances.

D. *Responses of normal and denervated submaxillary glands to pilocarpine.* The secretion of normal and denervated submaxillary glands in response to pilocarpine was tested in 7 animals. In 6 of the 7, the responses of the chronically denervated were 100 per cent or more greater than those of the control gland. The sensitization was equally striking in all 6 animals. The one animal in which pilocarpine had equal effects on the two sides failed to show any sensitization to adrenaline and acetylcholine. Figure 3 is part of the record from one of the positive experiments.

E. *Effect of cocaine on responses of normal and denervated submaxillary glands.* The effect of cocaine on salivary secretion in normal and denervated glands was tested in 3 animals. Intravenous injection of cocaine alone (5 to 7 mgm. per kgm.) did not excite salivary secretion in either acutely or chronically denervated glands. Its most striking effect was to make nearly equal the pilocarpine responses of the normal and sensitized glands. This was accomplished by increasing the secretion from the normal in response to pilocarpine while the secretion of the chronically denervated was increased only very slightly (fig. 3B). The responses to adrenaline were slightly increased after cocaine, more so on the normal than on the chronically denervated side. The responses to acetylcholine were not affected by cocaine in one animal in which records were taken before and after injection of this drug (7 mgm. per kgm.).

Discussion. Ide'i (1934) obtained histological evidence that the submaxillary gland of the rabbit, denervated 30 days previously by cutting the cervical sympathetic, responded more actively than the normal gland to adrenaline injected subcutaneously. His observations accord with the results reported above, in which the secretion due to adrenaline was increased after chronic sympathetic denervation. Fröhlich and Loewi (1910) presented a single protocol from an experiment (cat) showing that adrenaline has equal effects on the normal and the chronically denervated submaxillary sides. This, however, may have been an exceptional case. Ide'i also found (1933) that sympathetic denervation may have a selective action on the "light" and "dark" cells of the submaxillary. The differences were demonstrable cytologically. It is possible, therefore, that the one group of cells might be sensitized more than, or even to the exclusion of the other by sympathetic denervation. The degree of sensitization, or its very detection, would then depend on the relative proportion of the two types of cells within the gland. The variability in the sensitization of the submaxillary gland to adrenaline, though slight, is in contrast with the uniform results obtained in sensitizing the smooth muscle of the nictitating membrane of the cat by sympathetic denervation. It is important to observe, however, that this smooth muscle differs from the gland in being of uniform composition and in being supplied only by the sympathetic division of the autonomic nervous system.

Sympathetic denervation renders the smooth muscle of the nictitating membrane more sensitive not only to adrenaline but also to acetylcholine (Rosenblueth, 1932). The submaxillary gland is less regularly sensitized to acetylcholine than to adrenaline. The explanation given above for the results with adrenaline applies equally well to the failures to demonstrate uniform sensitization to acetylcholine. A second factor may have played a part in the inconstancy of sensitization to acetylcholine. Rawlinson (1933) showed that parasympathetic stimulation of the submaxillary gland of the cat through the *chorda tympani* caused changes predominantly in the alveolar cells. Stimulation of the gland through the cervical sympathetic or by means of adrenaline induced cytological changes principally in the demilune cells. It is possible that acetylcholine, a parasympathomimetic substance, may similarly affect especially the alveolar cells. This would render still less effective, for acetylcholine, the sensitizing action of sympathetic denervation. Pierce and Gregersen (1937) found a decrease in the secretory response to acetylcholine in the submaxillary of the dog denervated by excision of the *chorda tympani* some time previously. This result is difficult to interpret without further data.

The responses of the submaxillary gland to pilocarpine were very consistently increased by sympathetic denervation. Of the 7 animals in which this drug was tested, only one failed to show the phenomenon. This cat showed no sensitization to any of the drugs used. Takakusu (1922) reported increased responses from pilocarpine in the submaxillary gland of the rabbit denervated by excision of the superior cervical ganglion. Similar results were obtained by Pierce and Gregersen (1937) in the dog after chronic parasympathetic denervation (by excision of the *chorda tympani*). Why the results should be most consistent with pilocarpine, which is neither a sympathetic nor a parasympathetic mediator, is not clear.

Cocaine increases the secretory response of the normal submaxillary gland to adrenaline. This was reported by Fröhlich and Loewi in 1910 and agrees with the enhancing effect of cocaine when smooth muscle is stimulated by adrenaline. Of further interest is the fact that cocaine has a very much smaller effect on the gland already sensitized by sympathetic denervation. The failure of the drug to increase the responses of the submaxillary gland to acetylcholine is in agreement with the observations of Cattell, Wolff and Clark (1934), who found that cocaine increased the responses of the submaxillary gland to sympathetic stimulation but not to parasympathetic stimulation. Rosenblueth (1932) likewise found that the drug sensitized the nictitating membrane of the cat to acetylcholine less than to adrenaline.

The data recorded above, namely, that the denervated submaxillary gland of the cat responds more actively than the normal to pilocarpine,

adrenaline and acetylcholine, are strong evidence that chronic sympathetic denervation renders the gland more sensitive to chemical stimuli. The sensitization is not referable to vascular effects or to a discharge of stored secretions (cf. Pierce and Gregersen, *loc. cit.*). The effects of denervation are probably exerted directly on the secretory cells themselves. The nature of the change whereby denervation renders an effector more sensitive to stimulation is not clear. Various theories have been proposed, and these have been discussed in another publication (Simeone, 1938b).

CONCLUSIONS

1. Chronic denervation of the submaxillary gland of the cat by excision of the superior cervical ganglion sensitizes the gland to pilocarpine, adrenaline and, less consistently, to acetylcholine (tables 1 and 2; figs. 1, 2 and 3A).

2. Cocaine increases the responsiveness of the normal (acutely denervated) gland, but not that of the gland already sensitized by chronic sympathetic denervation (fig. 3B).

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pH OF THE CEREBRAL CORTEX AND ARTERIAL BLOOD UNDER INSULIN[1]

CLYDE MARSHALL, WARREN S. McCULLOCH AND LESLIE F. NIMS

From the Laboratories of Neuro-anatomy, Neurophysiology and Physiology, Yale University School of Medicine

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Insulin is known to produce changes in the chemistry of the blood and in the activity of the cortex. Moreover, it has been demonstrated that the "activity" of the cortex affects and is affected by its pH (1, 2). The question then naturally follows: does insulin alter the pH of the cortex and, if so, is this alteration primary in the cortex, or secondary to changes of pH in the arterial blood?

By means of the glass electrode techniques already described (2, 3, 4, 5) continuous records of pH were obtained from the cortex and arterial blood of animals curarized, constantly ventilated and injected with insulin.

Immediately following the intravenous injection of insulin no significant changes appear in the pH of the cortex, but after an interval of an hour to an hour and a half the pH of the cortex shows striking fluctuations having an abrupt rise sometimes preceded and always followed by a slower fall and recurring at short intervals, during which the pH is relatively stable. After intravenous injection of buffered dextrose solution these fluctuations do not recur and the cortex becomes more "acid." Figure 1 shows the changes in the pH of the cortex in a curarized monkey under constant artificial respiration after injection with insulin at 2:43 p.m. To be noted are the initial steadiness of pH (2:46), the four successive fluctuations (3:38, 3:59, 4:09, and 4:24) and the decline in pH after intravenous injection of dextrose (4:39).

Though the pH of the arterial blood after injection of insulin is not constant, it has no fluctuations corresponding to those of the cortex. Dextrose injected intravenously lowers first the pH of the arterial blood and subsequently that of the cortex. Figure 2 shows the pH of both arterial blood and cortex in a curarized dog under constant artificial respiration before and after injection of insulin at 2:19 p.m., the absence of any significant changes of the pH up to 3:45 whereafter three fluctuations of the pH of the cortex appear without corresponding changes of that of the arterial blood and, finally, the lowering of the pH of both by intravenous injection of buffered dextrose (4:14).

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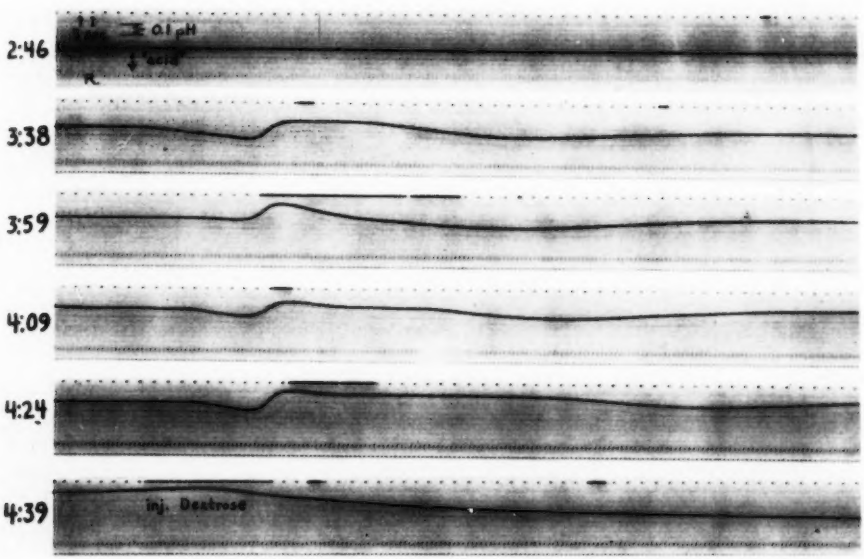

Fig. 1. Experiment of July 7, 1937. 8:00 a.m.: Macaca mulatta, 3 kgm. operated under ether. Exposure of right hemisphere; tracheal cannula. 9:56 a.m.: 5 cc. 1 per cent curare intramuscularly; artificial respiration. Recording of the pH of a locus of the sensorimotor cortex. 2:43 p.m.: 100 clinical units insulin (Lilly) by vein. 4:40 p.m.: 15 cc. of a 50 per cent buffered dextrose solution by vein. Time of excerpts from original continuous record indicated at left.

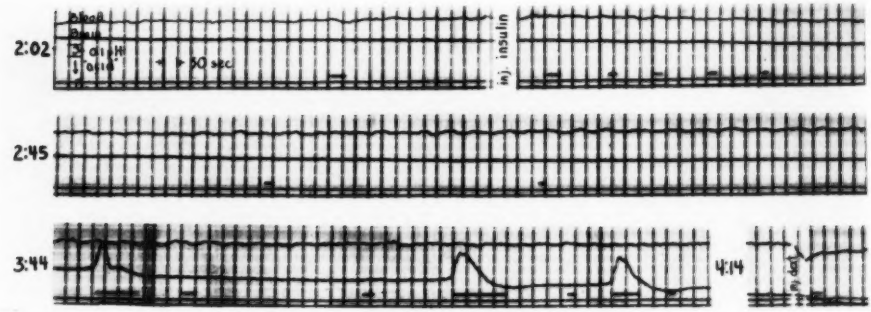

Fig. 2. Experiment of June 24, 1938. 9:45 a.m.: Dog 7.9 kgm. operated under ether. Exposure of right hemisphere and left femoral artery; tracheal cannula. 10:26-32 a.m.: 14 mgm. curare intravenously; artificial respiration (100 cc./32 per min.). 12:15 a.m.: 10 cc. 10 per cent chlorazol fast pink by vein. Blood pH cannula inserted. Recording of pH of cerebral cortex and arterial blood. 2:19 p.m.: 300 clinical units of insulin (Lilly) by vein. 4:14 p.m.: 10 cc. of a 50 per cent buffered dextrose solution by vein. Time of excerpts from original continuous record indicated at left.

Slow drifts of pH of both arterial blood and cortex occur in curarized animals (monkeys and dogs) following the injection of insulin, but these vary in magnitude and are inconstant in direction. On the other hand, the abrupt fluctuations of the pH of the cortex described above are a constant phenomenon. While they indicate some rapid alteration of active processes in the cortex, their chemistry is unknown; hence further interpretation is as yet impossible.

Fluctuations of the pH of the cortex of non-curarized, lightly narcotized animals injected with insulin appear concurrently with the convulsive seizures, but the intense muscular activity, involving changes in metabolism and respiration, so complicate the phenomena as to make analysis difficult. Nevertheless, even under these conditions these same phenomena can be detected and their concurrence with the seizures indicates that the abrupt changes of the pH of the cortex in the curarized animal reflect at the cortex one aspect of those central neural discharges which in the non-curarized animal result in motor seizures. In a previous paper (6) similar changes of the pH of the cortex, also without parallel changes of the arterial blood, have been shown to be associated with the cortical components of central neural discharges induced by intravenous injection of monobromide of camphor in the curarized animal.

The similarity of the changes of the pH of the cortex produced by these two convulsants in the absence of a change of the pH of the blood is of interest since the modes of action of insulin and monobromide of camphor in producing convulsions cannot be identical (hypoglycemia versus hyperglycemia; differences in the central discharges and motor seizures).

SUMMARY

1. One to one and one-half hour after intravenous injection of insulin into the curarized animal under constant artificial respiration fluctuations of pH of the cortex appear.

2. These fluctuations occur without corresponding fluctuations of pH of the arterial blood.

3. Intravenous injection of buffered dextrose solution lowers the pH of both arterial blood and cortex, and stops the recurrent fluctuations of pH of the cortex.

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THE INHIBITION OF BLOOD CLOTTING: AN UNIDENTIFIED SUBSTANCE WHICH ACTS IN CONJUNCTION WITH HEPARIN TO PREVENT THE CONVERSION OF PROTHROMBIN INTO THROMBIN[1]

K. M. BRINKHOUS, H. P. SMITH, E. D. WARNER AND W. H. SEEGERS

From the Department of Pathology, State University of Iowa, Iowa City

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In 1918 Howell and Holt (1) described a newly discovered anticoagulant, "heparin," which was found to intensify the ability of plasma to destroy thrombin. They also reported experiments which indicated that heparin had an inhibitory influence on the conversion of prothrombin into thrombin. The rôle of heparin in destroying thrombin has received general acceptance, but the "antiprothrombic" rôle has not been accepted by all workers. Particular difficulty was provided by the observations of Mellanby (2) and of Quick (3) that purified prothrombin is readily converted into thrombin in the presence of heparin. It is the purpose of the present article to show that these observations are, nevertheless, compatible with the dual rôle of heparin observed by Howell and Holt. We shall show that the inhibition of thrombin formation by heparin requires the presence of a plasma factor not previously recognized. This factor alone is not very effective as an "antiprothrombin," and heparin alone appears to have absolutely no effect in this regard. The two in conjunction, however, are highly effective in preventing the formation of thrombin. The new factor has not been identified, but we can show that it is abundantly present in plasma and in serum. It is apparently not diffusible, for it is to be found in serum which has been dialyzed against normal saline.

The reagents used were prepared as follows: 1. Serum. Dog blood was allowed to clot in a clean glass tube. After allowing to stand 1 hour at room temperature, the serum was obtained by centrifugalization. It was then dialyzed 4 hours against saline at 5° to remove diffusible materials. The serum contained only traces of residual prothrombin and thrombin. 2. Oxalated plasma, lung extract, and fibrinogen were prepared as previously described (4). 3. Calcium. Dissolve 0.15 gram NaCl and 1.0 gram $CaCl_2$ in 100 cc. water. 4. Rabbit brain extract. Grind 1 part of fresh washed rabbit brain with 9 parts of saline. Allow to stand

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several hours, centrifugalize, and retain the milky supernatant fluid. 5. Heparin (Hynson, Westcott and Dunning). One milligram prevents 2.5 cc. dog blood from clotting in 12 hours at room temperature. Dissolve 5 mgm. per cc. in saline, and dialyze 4 hours against saline. 6. Prothrombin was prepared in a purified state by the method of Seegers, Smith, Warner and Brinkhous (5). 7. Acacia. Fifteen per cent calcium free acacia in saline. 8. Imidazole buffer. Dissolve 1.72 gram imidazole in 90 cc. of 0.1 N HCl and dilute to 100 cc. This preparation is isotonic with saline (0.9 per cent NaCl) and has been found to be a highly efficient buffer at the pH used (7.25). It does not unite with calcium ion, and is therefore an ideal buffer for use in blood clotting studies (6).

Quantitative prothrombin determinations were made by the method developed in this laboratory (4, 7). In heparinized plasma the anti-

TABLE 1

Inhibition by heparin of the prothrombin conversion in oxalated plasma

TUBE NUMBER	OXALATED PLASMA	HEPARIN SOLUTION	CALCIUM	THROMBOPLASTIN BEEF LUNG EXTRACT (DIL. 1:30)	SALINE	CLOTTING TIME	UNCONVERTED PROTHROMBIN AT END OF 40 MINUTES INCUBATION
	cc.	*cc.*	*cc.*	*cc.*	*cc.*		*per cent*
1	2.0	0.0	1.2	0.2	0.6	30 sec.	4
2	2.0	0.0	0.0	0.0	2.0	No clot	100†
3	2.0	0.4	1.2	0.2	0.2	No clot 40 min.	98
4	2.0	0.4	1.2	0.2*	0.2	3 min.	22

* Undiluted.

† Control.

thrombic activity often interferes with the titration of prothrombin. To avoid this, one can precipitate the prothrombin with ammonium sulphate and thus eliminate the antithrombin in large part. This technic, described in detail elsewhere (7), was employed in the experiments given in table 1.

EXPERIMENTS. Tube 1, table 1, shows that recalcified plasma clots in 30 seconds if treated with moderate quantities of thromboplastin. The thrombin formed is destroyed by antithrombin almost as rapidly as it is produced, and within 40 minutes the serum is almost entirely devoid of both prothrombin and thrombin. The prothrombin titer, given in the last column of the table, was then only 4 per cent of that in the oxalated control (tube 2).

Tube 3 is identical with tube 1, except that heparin was included in the mixture. The failure to clot was clearly not the result of undue destruc-

tion of thrombin, for the persistence of prothrombin in undiminished amounts shows quite clearly that thrombin formation was entirely in abeyance.

This action of heparin can be neutralized by adding sufficient thromboplastin. In tube 4 the thromboplastin was increased 30-fold, but even this enormous amount did not abolish the heparin effect altogether, for 22 per cent of the prothrombin still persisted after a 40 minute period of incubation. It is our experience that at least twice this amount of thromboplastin is needed to effect complete conversion of the prothrombin into thrombin, and even then the conversion occurs more slowly than when heparin is absent.

It is thus seen from table 1 that, in whole plasma, heparin prevents completely the conversion of prothrombin into thrombin. To neutralize this effect of heparin, enormous quantities of thromboplastin are required.

TABLE 2

Conversion of purified prothrombin in the presence of heparin

TUBE NUMBER	PROTHROMBIN	SERUM	HEPARIN SOLUTION	CALCIUM	ACACIA	THROMBOPLASTIN (RABBIT BRAIN)	SALINE	IMIDAZOLE BUFFER	THROMBIN AT END OF 2 HOURS	UNCONVERTED PROTHROMBIN AT END OF 2 HOURS
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*units*	*units*
1	0.6	0.0	0.0	0.3	0.2	0.3	1.1	0.1	363	0
2	0.6	0.0	0.3	0.3	0.2	0.3	0.8	0.1	343	0
3	0.6	0.8	0.3	0.3	0.2	0.3	0.0	0.1	0	343
4	0.6	0.8	0.0	0.3	0.2	0.3	0.3	0.1	<1	52

Table 2 is presented to show that the inhibition involves an unknown plasma factor in addition to the known factors of the reaction—prothrombin, thromboplastin, calcium, and heparin. The prothrombin solution used in table 2 was prepared by the technic developed in this laboratory (5). It is a highly potent product, but in the process of purification it appears to be altered to some extent, for its transformation into thrombin occurs more slowly than in native plasma. In the experiments here reported the conversion was largely complete in a few minutes, but to effect complete conversion 2 hours were allowed. To stabilize the pH during this period of incubation, a small amount of imidazole was added as a buffer. We also added a small amount of 15 per cent acacia, for it is our experience that purified clotting reagents react more effectively and uniformly if acacia or some other inert colloid is added.

Tube 1, table 2 is a control tube showing the effect of incubating prothrombin, thromboplastin and calcium. These purified reagents contain practically no antithrombin, and hence the thrombin formed is not de-

stroyed. The final thrombin titer, at the end of 2 hours, was 363 units per cubic centimeter. The prothrombin had by then entirely disappeared, for no more thrombin could be produced.

Tube 2 of this table contained these same reagents, but it contained heparin in addition. Thrombin formation occurred essentially as though no heparin whatever were present. The purified reagents lack some plasma factor which is essential to the inhibition of thrombin formation, and without this factor heparin is unable to block the formation of thrombin. Direct support for this contention is supplied by tube 3, in which serum was added to supply the missing factor. This factor in conjunction with heparin blocked thrombin formation completely. At the end of two hours the prothrombin was still present in the original amount.

The serum factor alone has but slight ability to prevent the conversion of prothrombin into thrombin. This is shown in tube 4 which contained serum but no heparin. During the two hour period of incubation all but 52 units of the prothrombin was converted into thrombin, and the thrombin formed was destroyed by antithrombin present in the serum. It seems likely that the slight inhibitory power possessed by serum alone may be associated with traces of heparin which are present normally in serum.

Discussion. There still remains much uncertainty regarding the nature of the reactions which lead to the formation of thrombin. As long as this uncertainty exists, there is little point in speculating as to how inhibitors succeed in blocking the formation of thrombin. We wish merely to point out that heparin does block the reaction, but only when the newly discovered plasma factor is also present. It is perhaps safe to assume that the two substances in combination serve either as an antiprothrombin or as an antithromboplastin. Either assumption would seem to agree with all the facts now available.

The nature of the new plasma factor is also a problem for further study. It is obviously not dialyzable, for the serum used in our experiments was subjected to prolonged dialysis against saline. It is conceivable that this factor is identical with the substance which, in the presence of heparin, is such a powerful antithrombin (i.e., the "pro-antithrombin" of Howell). If this hypothesis is correct the substance in question might be defined as one which together with heparin not only prevents the formation of thrombin but also destroys any thrombin which may be present. This hypothesis, assuming identity for the two, has the advantage of simplicity, but it lacks positive proof, and we offer it merely as a suggestion.

SUMMARY

The effect of heparin in blocking the conversion of prothrombin into thrombin requires the presence in plasma of an accessory inhibitory factor

not previously recognized. Heparin alone does not block the formation of thrombin and the new factor alone has very little inhibitory power. However, the two together are highly effective in doing so. The new plasma factor is shown to be non-dialyzable but, aside from this, its identity has not yet been established.

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THE EFFECT OF CHEMICALS ON COCHLEAR POTENTIALS[1]

EDWARD M. WALZL

From the Otological Research Laboratory, The Johns Hopkins University

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The effect of salts, drugs and other chemicals on cochlear potentials has been studied by several investigators in attempts to throw more light on the relation of these potentials to actual hearing, and to determine the mechanism and structures involved in their production. Adrian, Bronk and Phillips (1) observed that injection of cocaine into the scala tympani through the round window membrane abolishes the cochlear response, and concluded that the potentials originate in excitable structures within the inner ear. Guttman (2) obtained similar results with several other drugs. Davis, Derbyshire, Lurie and Saul (3) found that application of crystals of cocaine to the round window membrane is just as effective as injecting solutions of the drug into the scala tympani. They found by this method that sodium chloride also abolishes the potentials. This was confirmed by Hallpike and Rawdon-Smith (4). A more extensive investigation of the effect of chemicals on cochlear potentials in cats and dogs was made by Fowler and Forbes (5), who observed that a variety of other substances (calcium chloride, glycerine and quinine di-hydrochloride), when applied to the round window membrane, cause progressive diminution in the response. They also observed that the response to high tones is impaired sooner and to greater extent than that to low tones. With the conditioned-reflex method, the application of sodium chloride to the round window membrane in dogs was found to produce a similar differential effect upon the hearing of high and low tones. Histological examination of cochleae subjected to this treatment revealed that the structures showing the greatest injury were the hair cells nearest the round window. Fowler and Forbes consider the coincidence of high tone loss with local destruction of the hair cells in the basal turn as evidence in support of the theory of tonal localization in the cochlea.

Wever and Bray (6) studied the effect on the cochlear response in the guinea pig of sodium chloride applied to the round window membrane. With stimuli of fixed intensity there was an initial augmentation of the response immediately after the salt was applied, quickly followed by a

[1] Aided by grants from the Ella Sachs Plotz Foundation and the Committee on Scientific Research of the American Medical Association.

drop for all tones, and only after about ten minutes did the differential effect on high and low tones appear. With stimuli of different intensities they found a relatively greater loss of response to loud than to faint sounds, and this differential effect was greatest for the low tones. These authors conclude that their results are not compatible with the contention of Fowler and Forbes that the action of sodium chloride is due simply to local destruction of the hair cells, but that several processes are involved, viz., change in electrical conductivity, disturbance in the pressure relations within the cochlea, and progressive impairment of the function of the hair cells.

Eyster, Bast and Krasno (7) pointed out that with the above methods the chemicals invariably come in contact with the structures in the middle ear, and they contend that the effects are due simply to impairment of the conductive mechanism.

The Wever and Bray phenomenon (8) has been used in many studies on the physiology of hearing, but these experiments can never be interpreted until we know exactly where cochlear potentials arise and their relation to hearing. The following experiments prove conclusively that cochlear potentials arise in some structure within the cochlear duct and not from either Reissner's or the basilar membrane.

MATERIAL AND METHODS. Cats anesthetized with urethane by stomach tube or phenobarbital sodium intraperitoneally were used. The animals were tracheotomized and a gentle stream of air maintained through a T-shaped tracheal cannula. The cochlea was exposed by opening the ventral surface of the bulla and removing the septum between the bulla and the middle ear. The cochlear potentials were picked up by silver electrodes, one on the mucosa near the rim of the round window niche and the other in the muscles of the neck, passed through a transformer-coupled amplifier and converted into sound with a head-phone.

The method of measuring the cochlear potentials is, with but slight modifications, the same as that used clinically to determine the threshold of hearing. The intensity of the stimulating tone is varied until the amplified cochlear potentials are just heard by the observer. Under control conditions, sounds at about average human threshold elicit a detectable response. In the present experiments five tones are used; they cover the frequency range from 256 to 4096 cycles per second at octave intervals. The tones are produced by a special audiometer, loaned by the Bell Telephone Laboratories, and the sound is led from the shielded 555-W receiver to the external meatus of the animal through a $\frac{3}{4}$ inch rubber hose. The control test of each ear is the base line for the experiment. After each experimental procedure the intensity of the sound required for a "threshold" response is determined. The difference between this reading and the control, as measured in decibels with the attenuator of the audiometer, is recorded as the effect of the experimental procedure.

Time alone, at least up to six or seven hours and with the anesthetics used, does not cause an impairment of cochlear potentials for any of the five tones.

Four methods have been used to introduce chemicals into the inner ear:

1. Crystals of NaCl or KCl are placed on the round window membrane.

2. Crystals of these salts are placed on the exposed endosteum of either the scala tympani or the scala vestibuli of the basal turn. The endosteum is exposed with a dental burr without damage to the membranous inner ear.

3. Crystals of the salts are introduced directly into the perilymph of the apical turn through a hole drilled into the scala vestibuli near the heli-

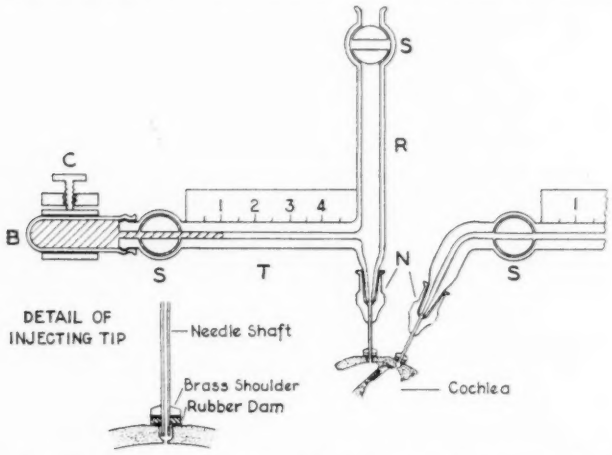

Fig. 1. Sectional diagram of the apparatus used in replacement of the perilymph of the cochlea. *B*, rubber bulb; *C*, clamp; *N*, hypodermic needles with modified tips; *R*, reservoir; *S*, stopcocks; *T*, calibrated capillary tube.

cotrema. The opening is immediately closed by a piece of rubber dam on the end of an applicator.

4. The perilymph of the entire cochlea is replaced by the solution to be tested. To permit total replacement, two holes are drilled into the lower part of the basal turn, one into the scala tympani, the other into the scala vestibuli. The amount of fluid injected and the amount escaping are measured. The inlet portion of the apparatus used for this purpose (fig. 1) consists essentially of a calibrated glass capillary tube, *T*, a reservoir, *R*, and a mercury-filled rubber bulb, *B*, with a screw clamp, *C*, for the control of the injection of the fluid. A syringe with a long needle is used to fill and to change the fluid in the reservoir. The outlet portion of the apparatus is a calibrated capillary tube with a stopcock. The inlet

portion of the apparatus is connected to the scala tympani, and the outlet portion to the scala vestibuli. The connections to the holes in the scalae are made with no. 22 hypodermic needles, each of which has a shoulder covered with rubber dam to seal the joint between the needle and the bone. The shoulder is made by soldering a perforated thin brass disc near the end of the needle. The part of the needle projecting beyond the shoulder is cut off to a length less than the thickness of the bone.

The holes into the scalae are made by first drilling a depression almost through the bone with a dental burr slightly larger in diameter than the needle, then flattening the bone around this depression in order to make possible a water tight contact with the shoulder of the needle, and lastly perforating the floor of the depression with a root canal drill. Perforation is determined by the appearance of a small drop of perilymph. Care must be taken to avoid the spiral ligament and the veins in the endosteum of the scala tympani.

Effect of local application of salts to the cochlea. Two of the methods (2, 3) by which salts are applied locally involve drilling a hole through the bony wall of the cochlea. It was therefore necessary to ascertain whether or not the operation alone affects the cochlear response. Accordingly, control experiments were run in which the only procedure was the drilling of the hole, after which readings were made at frequent intervals throughout the day. The average change was only +2 db. for eight experiments in which the endosteum of either the scala tympani or the scala vestibuli was exposed, and only +1 db. for nine experiments in which a hole was drilled into the apex. However, to make sure in individual experiments that no damage had been done, considerable time was allowed to elapse between the drilling and the application of the salt. If there was a change in the cochlear response it indicated intracochlear bleeding or other damage and the experiment was discontinued.

The first two experiments in table 1 are typical of the effects of local application of NaCl and KCl to the basal coil. Application of crystals of these salts to the round window membrane or to the exposed endosteum of the basal coil caused marked impairment for the high tones, and considerably later, a slight impairment for the low tones. In most of the experiments of this type there was some improvement, usually about five db., for the low tones preceding their fall.

The third experiment presented in this table shows that when crystals of a salt were put into the apex, the first effect was exactly opposite to that obtained by applying the salt to the basal coil, i.e., rapid impairment for the low tones. Later there was impairment for all tones. In general, the differential effect on low tones obtained by putting salt into the apex was much less than that on high tones obtained by applying the salt to the basal coil.

When a salt is applied to the basal coil of the cochlea, the diffusion gradient is from base to apex, and when put into the apex it is in the reverse direction, i.e., from apex to base. Since with the former method the high tones are the first to show an impairment, and with the latter the low tones are the first to be affected, it is reasonable to conclude that the structures involved in threshold response to high tones are located toward the base, and those involved in threshold response to low tones are located toward the apex. These conclusions are in general agreement with those of Fowler and Forbes.

TABLE 1

Effect of local application of sodium chloride to the cochlea

PROCEDURE	TIME AFTER INDICATED PROCEDURE	CHANGE IN THRESHOLD FROM CONTROL READING				
		Frequency				
		256	512	1024	2048	4096
	min.	*decibels*	*decibels*	*decibels*	*decibels*	*decibels*
5 crystals* of NaCl placed on the round window membrane	5	−5	−5	−5	+5	+5
	20	0	−5	0	+25	+20
	40	0	0	0	+20	+50
	94	+20	+15	+10	+60	+85
Hole through bone over scala vestibuli of basal turn. Endosteum intact	90	+5	+5	0	0	+5
3 crystals of NaCl placed on hole	5	0	+5	0	0	+15
	22	+15	+15	+15	+15	+45
	60	+15	+15	+25	+20	+70
Hole into scala vestibuli of apex	70	+5	0	−5	0	+5
6 crystals of NaCl put into apex and hole closed	4	+25	+10	+5	+5	0
	42	+55	+60	+45	+45	+50

* The crystals used weigh about 0.1 mgm. each.

Replacement of the perilymph. Although the above experiments are of value for producing local damage, it is not evident from these results whether the effect obtained is due to a specific action of the substance itself or to mechanical damage by osmotic shrinkage. Also, the concentration of the salt in each region of the cochlea is unknown. The ideal method of testing the action of substances is to apply them in solutions of known concentration and composition. Accordingly, in the following experiments, the effects of changing the concentration of some of the normal constituents of the perilymph were studied by replacing the perilymph with various perfusion solutions.

In order to learn whether or not perfusion *per se*, or its attendant opera-

tive procedures (drilling holes into the perilymphatic scalae, etc.) impair the cochlear response, fifteen control experiments were performed in which the perilymph was replaced with cerebro-spinal fluid. Cerebro-spinal fluid was selected as the control perfusion fluid since it is probably identical with perilymph, due to the anatomical continuity of the perilymphatic and the subarachnoidal spaces through the cochlear aqueduct. The results of these control experiments are presented in table 2, which shows that, with but one exception, the maximum change for any tone was ±5 db., and the average change for all the experiments, less than +1 db. This is within the limits of error of the method.

TABLE 2

Effect on the cochlear response in fifteen experiments of drilling a hole into the scala vestibuli and one into the scala tympani of the basal turn, and replacement of the perilymph with cerebro-spinal fluid

VOLUME OF CEREBRO-SPINAL FLUID PERFUSED	TIME AFTER START OF PERFUSION	CHANGE IN THRESHOLD FROM CONTROL READING				
		Frequency				
		256	512	1024	2048	4096
*mm.*³	*min.*	*decibels*	*decibels*	*decibels*	*decibels*	*decibels*
16	90	0	0	−5	−5	−5
125	80	0	0	+5	0	+5
16	22	+5	0	+5	+5	0
250	23	−5	+5	−5	0	0
125	23	0	−5	0	0	+5
250	69	−5	−5	−5	0	−5
125	76	+5	0	0	0	+5
250	70	0	0	+5	+5	0
500	106	0	0	0	0	+5
250	150	0	0	0	0	0
250	72	−5	−10	+5	0	0
250	36	+5	+5	−5	0	−5
250	96	−5	0	0	0	0
250	91	0	0	−5	0	−5
1250	110	0	−5	0	0	0

In all experiments the cochlea was first perfused with cerebro-spinal fluid, and if there was no loss in the response during the first hour it was assumed that no intracochlear damage had resulted from the operative procedure, and the cochlea was then perfused with the solution to be tested. Only the results of experiments in which the loss from perfusion with cerebro-spinal fluid was insignificant are reported below.

Replacement of the perilymph with isotonic soluions of NaCl, KCl, and $CaCl_2$. The first three experiments recorded in table 3 are typical of the experiments in which the perilymph was replaced by isotonic solutions of NaCl, KCl, and $CaCl_2$. Perfusion with these solutions caused a relatively

TABLE 3

Effect on the cochlear response of replacement of the perilymph with various solutions

PROCEDURE	TIME AFTER INDICATED PROCEDURE	CHANGE IN THRESHOLD FROM CONTROL READING				
		Frequency				
		256	512	1024	2048	4096
	min.	*decibels*	*decibels*	*decibels*	*decibels*	*decibels*
Holes in scala tympani (s.t.) and scala vestibuli (s.v.) and perfusion with cerebro-spinal fluid (CSF)	27	−5	+5	−5	0	0
Perf. with 25 mm³ of 0.7% NaCl	20	+15	+20	+25	+15	+20
Perf. with CSF	10	+10	+15	+5	+5	+10
Perf. with 25 mm³ of 0.7% NaCl	28	+30	+30	+20	+15	+20
Holes in s.t. and s.v. and perf. with CSF	80	+5	0	0	0	+5
Perf. with 25 mm³ of 0.88% KCl	5	+20	+10	+10	+10	+20
	65	+50	+35	+30	+30	+50
Holes in s.t. and s.v. and perf. with CSF	71	0	0	−5	0	−5
Perf. with 50 mm³ of 1 vol. 2.6% $CaCl_2$ plus 1 vol. CSF	42	+5	+5	+5	+10	+5
Perf. with 50 mm³ of 2.6% $CaCl_2$	5	+10	+10	+10	+15	+10
	26	+25	+30	+30	+35	+30
Holes in s.t. and s.v. and perf. with CSF	70	0	0	+5	+5	0
Perf. with 175 mm³ of 2.2% dextrose	28	+15	+15	+15	+20	+20
Perf. with CSF	106	+5	+5	0	0	+5
Holes in s.t. and s.v. and perf. with CSF	150	0	0	0	0	0
Perf. with 100 mm³ of 1 vol. CSF plus 1 vol. H_2O	22	−5	0	0	0	0
Perf. with 50 mm³ of 1 vol. CSF plus 3 vols. H_2O	28	+30	+30	+25	+25	+30
Holes in s.t. and s.v. and perf. with CSF	80	0	0	0	0	+5
Perf. with 2.6 cc of artificial CSF of 2 times normal conc.	20	+5	0	+5	0	+5
Perf. with 2 cc of artificial CSF of 4 times normal conc.	20	+5	0	+5	0	+5
Perf. with 2 cc of artificial CSF of 10 times normal conc.	5	+25	+25	+20	+15	+20
Holes in s.t. and s.v. and perf. with CSF	34	−5	0	0	0	0
Perf. with 50 mm³ of 1 vol. 0.6% $NaHCO_3$ plus 9 vols. CSF (pH = 8.5)	30	−5	0	0	0	0
Perf. with 50 mm³ of 2 vols. 0.6% $NaHCO_3$ plus 8 vols. CSF (pH = 9.4)	23	0	0	+5	0	+5
Perf. with 50 mm³ of 4 vols. 0.6% $NaHCO_3$ plus 6 vols. CSF (pH = 10.0)	22	+10	+20	+15	+10	+15
Perf. with 50 mm³ of 6 vols. 0.6% $NaHCO_3$ plus 4 vols. CSF (pH = 10.5)	4	+15	+20	+15	+15	+20

TABLE 3—*Concluded*

PROCEDURE	TIME AFTER INDICATED PROCEDURE	CHANGE IN THRESHOLD FROM CONTROL READING				
		Frequency				
		256	512	1024	2048	4096
	min.	*decibels*	*decibels*	*decibels*	*decibels*	*decibels*
Holes in s.t. and s.v. and perf. with CSF	130	0	−5	0	0	0
Perf. with 50 mm³ of 1 vol. M/10 HCl plus 3 vols. CSF (pH = 6.4)	28	+10	0	+10	+5	0
Perf. with 150 mm³ of 1 vol. M/10 HCl plus 2 vols. CSF (pH = 3.4)	80	+25	+15	+20	+15	+15

gradual impairment of the cochlear response. The rate and the extent of the impairment in any one experiment were approximately the same for all five tones. The effect of solutions of KCl or of $CaCl_2$ is much greater than that of solutions of NaCl. This was especially clear in one experiment in which the cochlea was perfused with an isotonic solution of NaCl preceding perfusion with isotonic KCl. With the former the threshold was impaired less than 5 db. after half an hour, while five minutes after perfusion with KCl there was an impairment of more than 20 db.

In several experiments the cochlea was perfused with an isotonic solution of $CaCl_2$ from base to apex, i.e., only the perilymph in either the scala tympani or the scala vestibuli was replaced. The results were essentially the same as those obtained by replacing the perilymph of the whole cochlea.

Replacement of the perilymph with isotonic solutions of dextrose and sucrose. The fourth experiment in table 3 is typical of those in which the perilymph was replaced with an isotonic solution of dextrose. Similar experiments were performed with sucrose. Perfusion with an isotonic solution of dextrose or sucrose caused a relatively gradual impairment in the threshold for all tones, usually 15 to 20 db. within thirty minutes. If at this time the solution was replaced with cerebrospinal fluid there was a gradual, and in some instances a complete, recovery to the control level.

Replacement of the perilymph with hypo- and hypertonic solutions. The fifth experiment recorded in table 3 is typical of those in which the perilymph was replaced with cerebro-spinal fluid made increasingly hypotonic by the addition of distilled water; the sixth in this table is an experiment in which the perilymph was replaced consecutively with solutions containing the same relative proportions of chemicals as cerebro-spinal fluid (0.4 per cent NaCl, 0.02 per cent KCl, 0.02 per cent $CaCl_2$ and 0.05 per cent dextrose), but with their concentrations increased two, four, and ten times the normal. Great changes in the tonicity of the perfusion fluid have but little effect on threshold. Only with very dilute solutions (one-

fourth normal or less) or with very concentrated solutions (ten times normal) were significant impairments obtained. As in the other perfusion experiments, all five tones were affected equally.

Replacement of the perilymph with solutions of different hydrogen-ion concentrations. The last two experiments in table 3 show, respectively, the results of a typical experiment in which the perilymph was replaced with cerebro-spinal fluid in which the hydrogen-ion concentration was progressively decreased by the addition of an isotonic solution of $NaHCO_3$, and one in which it was increased by adding HCl. In this group of experiments almost no effect on cochlear response was caused by a change of more than two pH units in the alkaline direction or a change of one pH unit in the acid direction from the normal for cerebro-spinal fluid. Greater changes caused a relatively gradual decrease in the cochlear response for all tones.

Discussion. There are three principal views with regard to the origin of cochlear potentials. Howe and Guild (9), and Davis et al. (3), on the basis of their observations that cochlear potentials cannot be elicited in deaf albino cats which lack an organ of Corti, conclude that the source of the potentials is in the organ of Corti. Hallpike and Rawdon-Smith (4) postulate that the potentials are developed across Reissner's membrane as a result of a chemical difference between endolymph and perilymph. Bast et al. (10), on the other hand, assume a passage of fluid through Reissner's membrane with each condensation and rarefaction phase of the sound wave, and believe that the cochlear potentials are simply streaming potentials.

If the cochlear potentials are streaming potentials, their magnitude should vary with the charge on the surface of the membrane. Changes in hydrogen-ion concentration are known to affect the charge on the surfaces of biological membranes. Since in our experiments on perfusion with cerebro-spinal fluid of different hydrogen-ion concentrations the cochlear response was only slightly affected within wide changes of pH, it seems unlikely that the cochlear potentials are streaming potentials. Furthermore, in all of our experiments in which the cochlea was perfused, the effects occurred relatively slowly. This is particularly significant in the case of perfusion with a non-electrolyte such as dextrose. Replacement of the perilymph on one side of Reissner's or of the basilar membrane with a non-electrolyte would cause an immediate change in the potential if it were either a streaming or a membrane potential dependent on an ionic difference between endolymph and perilymph. These facts, together with the high tolerance for changes in tonicity shown in the experiments on perfusion with hypo- and hypertonic solutions, lead us to the conclusions that the structures giving rise to the cochlear potentials are not in immediate contact with the perilymphatic scalae, and that the

potentials are neither streaming nor concentration potentials across the membranes separating the scala media from the perilymphatic scalae. Our results do agree, however, with the view that some structure in the scala media produces the potentials.

Most theories of hearing assume a localization of tones to specific regions of the cochlea. Tonal localization for cochlear potentials is indicated by the experiments of Hallpike (11), Culler (12), and others with electrodes at different points on the surface of the cochlea, by the experiments of Fowler and Forbes (5) on salt applied to the round window membrane, and by all of our experiments in which a localized application of a salt was made, whether to the round window membrane, to the exposed endosteum of the basal turn, or by placement into the perilymph of the opened apex. None of these experiments, however, give any information concerning the completeness or sharpness of the localization.

The question has frequently been raised as to whether or not cochlear potentials are manifestations of cellular activity or simply the result of gross movements of some structure in the inner ear. Probably the best evidence that the former is true is that there is a rapid decrease in the potential with ischemia of the cochlea due to cutting of the internal auditory artery or the death of the animal. It may be contended, however, that the attendant fall in blood pressure causes a loss of turgidity which results in simple mechanical change of the parts responsible for the production of the potentials. In our experiments with isotonic solutions the only effect is a chemical one. A specific, or toxic, action of the chemical used must be responsible for the effects observed in these perfusion experiments. No other interpretation can explain the different degrees of impairment obtained by the use of isotonic solutions, especially in view of the fact that perfusion with cerebro-spinal fluid causes no impairment. These observations indicate strongly that the cochlear potentials depend on the metabolic integrity of some tissue or group of cells.

Our experiments demonstrate that these cellular elements must be within the cochlear duct and cannot be part of any of the structures in direct contact with perilymph. These cells must also be so located as to explain the differential impairment of the response to high and low tones. The only reasonable conclusion from these requirements is that the organ of Corti, which is accepted as the end-organ of hearing, is also the source of cochlear potentials.

SUMMARY

Additional information with respect to the source of cochlear potentials has been obtained by studying the effects of salts applied locally to the cochlea of cats and by replacing the perilymph with various solutions.

When crystals of NaCl or of KCl were applied locally to the basal coil

the first effect was impairment of response to high tones and when applied to the apical coil the first effect was on low tones. With both of these methods there was later impairment for all tones.

Replacement of the perilymph was made with a special apparatus, which is described. Fifteen control experiments, in which the cochlea was perfused with cerebro-spinal fluid, showed that the operative procedures involved do not affect cochlear potentials. The effect of perfusion with the following solutions was studied: 1, isotonic solutions of NaCl, KCl and $CaCl_2$; 2, isotonic solutions of dextrose and sucrose; 3, hypo- and hypertonic cerebro-spinal fluid, and 4, cerebro-spinal fluid with increased or decreased hydrogen-ion concentrations. With all of these solutions the impairments of cochlear potentials which resulted were equal for all tones. There was, however, a great difference in the time before onset of impairment and in the total impairment caused by the different solutions.

The following conclusions were drawn from these experiments: 1, the effect of a salt on cochlear potentials is due to chemical action and not to osmotic damage; 2, the cochlear response to high tones is localized toward the basal end of the cochlea and that for low tones, at least to some extent, toward the apex; 3, cochlear potentials are neither streaming nor concentration potentials across either of the membranes that separate the scala media from the perilymphatic scalae; 4, the structures giving rise to the cochear potentials are in the scala media, and are probably the hair cells of the organ of Corti.

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ADRENALIN AND THE METABOLISM OF PERIPHERAL TISSUES

LEONARD CAMMER AND FRED R. GRIFFITH, JR.

From the Department of Physiology, University of Buffalo, Buffalo, New York

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This is to report the effect of intra-arterial administration of adrenalin on blood flow and respiratory exchange of the resting, intact, hind leg of the cat.

METHODS. The cats were anesthetized with chloralose, 0.1 gram per kilo, given subcutaneously. As soon as anesthesia was complete, a tracheal cannula was placed, and the aorta and inferior vena cava exposed at their bifurcations through a ventral, midline incision. Leaving the circulation of the left leg intact, the sacral and right iliac arteries were tied off, the right iliac (femoral) vein cannulated at its central end, and a loose ligature placed beneath the inferior vena cava. Traction on this ligature diverts all blood from the (intact) left leg into the central cannulated stump of the right femoral vein, where it can be measured and sampled in a manner somewhat similar to that described by Himwich and Castle (1927); a simultaneous arterial sample is obtained by syringe from a fine needle inserted into the central stump of the right iliac artery to the bifurcation of the aorta. The latter also provided means for intra-arterial injection of saline or adrenalin intended for distribution to the left leg.

Forty-eight experiments were performed. Of these, ten were uninjected controls; ten others received only 0.9 per cent NaCl in amount equal to that serving as vehicle for the adrenalin which was administered to the remaining twenty-eight in various concentrations, as given below.

Each experiment consisted of the taking of four arterial-venous pairs of samples at ten-minute intervals, thus delimiting a half-hour period. When injection was made, either of isotonic NaCl or adrenaline, it was begun five minutes after the second sampling, continued exactly five minutes, and the third pair of samples secured immediately upon its completion, i.e., exactly ten minutes after the previous (second) blood sampling. Ten minutes later the fourth and last pair of samples was secured.

Injection. Neutral (pH 7.3), 0.9 per cent NaCl was used for injection and dilution of Parke-Davis, 1:1000 adrenalin chloride, to provide rates of administration, based on total body weight, of 0.002 (7 expts.), 0.000,4 (10 expts.), 0.000,002 (5 expts.) and 0.000,000,2 (6 expts.) mgm. per kilo

per minute. Dilution was made only immediately before use. Injection, either of saline or adrenalin, was from a 5 cc. syringe, by hand, 1 cc. per minute for 5 minutes.

Blood analysis. Oxygen capacity for estimation of change in blood concentration was determined by the colorimetric method of Cohen and Smith as modified by Wu (1922) for alkaline hematin. Oxygen and carbon dioxide contents were obtained by the manometric method of Van Slyke and Neill (1924) using 0.2 cc. samples in duplicate. Proper precautions were taken to prevent alteration in the gas content of the blood samples awaiting analysis.

RESULTS. *Adrenalin concentrations.* Although no effort was made to keep the concentration of adrenalin reaching the tissues of the injected leg exactly constant, the weights of the animals and the normal rates of blood flow were sufficiently uniform so that rate of injection was in each instance the factor chiefly determining concentration. Thus, using average figures, the initial concentrations of adrenalin reaching the tissues of the injected leg were, for each of the rates of injection used, approximately as follows:

0.000,000.2	mgm. per kilo per minute	$1 \times 10^{-10.6}$
0.000,002	mgm. per kilo per minute	$1 \times 10^{-8.6}$
0.000,4	mgm. per kilo per minute	$1 \times 10^{-7.2}$
0.002	mgm. per kilo per minute	$1 \times 10^{-6.4}$

When adrenalin injection decreased blood flow, as with the three highest rates of injection used, the final concentration reaching the tissues would be proportionally increased. The above values, therefore, merely indicate the approximate concentrations responsible for initiating changes in blood flow and respiratory exchange when these were affected.

Oxygen capacity and blood concentration. Calculation of tissue respiratory metabolism, from determination of arterial-venous blood-gas contents and rate of blood flow, necessitates consideration of change in blood concentration, if any, as it passes through the tissue. Evidence regarding this was obtained by determination of hemoglobin content, or oxygen capacity, of the respective arterial-venous samples. Averages for the first and second pairs of normal samples for the entire forty-eight experiments were:

First arterial sample	19.95 vol. per cent
First venous sample	19.70 vol. per cent
Difference	−1.25 per cent
Second arterial sample	20.30 vol. per cent
Second venous sample	19.70 vol. per cent
Difference	−2.95 per cent

A similar decrease in concentration also occurred between the arterial-venous pairs of the third and fourth samples of the normal controls (10 expts.) and saline-injected animals (10 expts.). Averages for these twenty experiments were:

Third arterial sample	19.70 vol. per cent
Third venous sample	19.65 vol. per cent
Difference	−0.25 per cent
Fourth arterial sample	20.00 vol. per cent
Fourth venous sample	19.30 vol. per cent
Difference	−3.50 per cent

On the other hand, injection of adrenalin reversed this and resulted in concentration of the venous blood; averages for the third and fourth pairs of samples, following adrenalin injection (28 expts.), were:

Third arterial sample	19.10 vol. per cent
Third venous sample	19.35 vol. per cent
Difference	+1.31 per cent
Fourth arterial sample	19.25 vol. per cent
Fourth venous sample	19.80 vol. per cent
Difference	+2.86 per cent

Although the indicated corrections are small (see also, Griffith and Hummel, 1930) they were applied to all calculations of the respiratory exchange according to the formulae of Himwich and Castle (1927), affecting absolute values only slightly and qualitative relationships and interpretation none at all.

Spontaneous changes. Blood flow: Reference to figure 1 shows that always there was slight decrease in flow rate between the first and second normals; for the entire forty-eight experiments average values at the beginning and end of this 10-minute interval are 21.7 and 19.0 cc. per minute, respectively. The control series (I, fig. 1) indicates that under these experimental conditions (blood sampling and other trauma) this decline continues with little change during the subsequent 20 minutes which included the period of injection and recovery for the remaining experiments. Injection of 0.9 per cent NaCl (II, fig. 1) was without significant effect.

Oxygen consumption. With the exception of the 10 experiments of the control series and the equal number of those subsequently receiving 0.000,4 adrenalin (I and V, fig. 1), there is spontaneous decline in oxygen consumption during the 10-minute interval between the first and second normal samples; average values for the entire 48 experiments are 1.29 and 1.21 cc. per minute respectively. This average normal parallelism

between declining blood flow and oxygen consumption is further evident during the subsequent 20-minute interval of both the control and saline series (I and II, fig. 1); thus indicating, again, that injection of saline, of itself, is without effect.

Carbon dioxide production. It is only necessary to examine the accompanying figure to see that normally carbon dioxide production follows fairly closely the declining trend of blood flow and oxygen consumption;

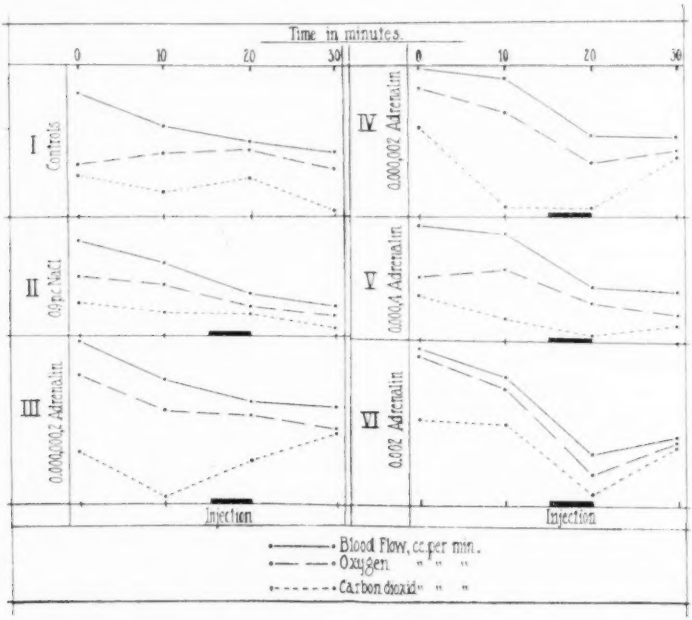

Fig. 1. Blood flow (continuous line), oxygen utilization (dashed line) and carbon dioxide output (dotted line) all in cubic centimeters per minute, of the intact, left hind-leg of chloralose anesthetized cats during a half hour period which included, except for the controls (I), injection of isotonic NaCl (II) and adrenalin in various concentrations (III, IV, V, VI).

average values for the first and second normals of the entire 48 experiments being 1.20 and 0.80 cc. per minute, respectively. Again it is evident that saline injection is without significant effect.

In summary, the evidence would lead to expectation of gradual, spontaneous and roughly-parallel decline in blood flow, oxygen consumption and carbon dioxide production during the half-hour period of these experiments. Also, injection of 0.9 per cent NaCl, in amount equal to that serving as vehicle for the subsequently injected adrenalin, is without significant effect.

The effect of adrenalin. Blood flow and oxygen consumption. These two are affected so similarly, they may be considered together. Using the controls and saline injections (I and II, fig. 1) for comparison, the lowest rate of injection (0.000,000,2 mgm. per kilo per minute; III, fig. 1) would appear without effect. Although it might be possible, by extrapolation of the slope established by the first and second normals, to interpret the oxygen curve as showing a less than expected rate of decline, comparison with the saline and uninjected controls makes it doubtful that the observed deviation is outside normally expected variation. At least, and this is probably the most significant conclusion from the evidence, injection of adrenalin at this rate did not, at any time, increase oxygen consumption above the pre-existing normal rate.

The three higher rates of injection all significantly decreased both blood flow and oxygen utilization in almost parallel degree. In spite of their 200-fold difference, 0.000,002 and 0.000,4 mgm. per kilo per minute produced almost identical average effects (IV and V, fig. 1); assumption of an all-or-none type of reaction, or that the lesser of these was already maximal, is untenable in light of the approximately 2-fold greater decrease resulting from injection at only slightly increased rate, 0.002 mgm. per kilo per minute (VI, fig. 1). Quantitation of this kind is, however, probably unwarranted by the data at hand; the only conclusion indubitably justified, and the only one which is of interest at the moment, is that effective concentrations of adrenalin appear to decrease, and never increase, blood flow and oxygen consumption under the conditions of these experiments.

Carbon dioxide output. Inspection of III, IV, V, and VI of figure 1 shows that adrenalin has a more diverse effect on carbon dioxide output from the leg than on blood flow or oxygen utilization. Nevertheless the effect can be seen to bear a consistent relation to dosage. The least rate of injection (0.000,000,2 mgm. per kilo per minute; III, fig. 1) produces unmistakable increase, persisting without interruption into the 10-minute interval following injection. As dosage is increased, the output at the end of injection is, first, merely kept from falling at the normal rate (0.000,002 mgm. per kilo per minute, IV, fig. 1); then, although it continues to decrease at approximately the normal rate during injection, the depression, at least, is not as great as that suffered by blood flow and oxygen consumption (V, fig. 1, 0.000,4 mgm. per kilo per min.); and, finally, with the maximal rate of injection (0.002 mgm. per kilo per minute, VI, fig. 1) depression of output parallels that of blood flow and oxygen consumption. In all cases output following injection is increased.

Since maximal increase of output occurs with the smallest employed dose and one which has no appreciable effect on oxygen consumption or blood flow, it becomes immediately doubtful if these alterations are related to cellular oxidation; this will receive further consideration in what follows.

Respiratory quotient. Table 1 summarizes the average values for each series.

TABLE 1

	1st NORMAL	2nd NORMAL	3rd SAMPLE	4th SAMPLE	NUMBER OF EXPERIMENTS
I. Controls	0.89	0.70	0.78 Immediately and 10 min. after injection	0.65	10
II. Saline-injected	0.76	0.72	0.84	0.78	10
Average of I and II	0.83	0.71	0.81	0.72	
III. 0.000,000,2 adrenalin	0.77	0.68	0.91	1.18	6
IV. 0.000,002 adrenalin	0.77	0.50	0.64	0.88	5
V. 0.000,4 adrenalin	0.84	0.65	0.69	0.86	10
VI. 0.002 adrenalin	0.68	0.81	0.79	1.02	7
Grand average	0.79	0.70			
Average after adrenalin			0.75	0.96	

If the third and fourth samples of the control and saline-injected series are included with the normals of all series, and all observations after adrenalin injection are grouped together, the total data may be reduced to the following statistics:

	NUMBER OF OBSERVATIONS	RANGE	MEAN	STANDARD DEVIATION
Normals	136	0.14–2.23	0.75 ± 0.02	0.32
After adrenalin	56	0.15–1.68	0.79 ± 0.03	0.31

This summary indicates that with adequate sampling the method is apparently adequate to establish metabolically significant quotients; and that the occurrence of unreasonable values in the averages of the individual series is attributable to paucity of determinations rather than abnormality of metabolism. Also, by either method of presentation there is evident a tendency for the quotient to be increased by adrenalin; although the difference, $0.79 \pm 0.03 - 0.75 \pm 0.02 = 0.04 \pm \sqrt{.03^2 + .02^2} = 0.04 \pm 0.036$, is apparently without statistical significance. This cannot be accepted as final answer to this perplexing problem but merely as another small contribution to its already large literature (Erichson).

Discussion. In spite of the admittedly large experimental error inherent in work of this kind, the reasonably close agreement and parallel variation of all normal and control observations and, on the whole, the

apparently consistent results of injection lead to confidence in the adequacy of the technique employed.

Insofar, therefore, as blood flow and oxygen utilization are concerned, the results indicate that any effective concentration of adrenalin diminishes both. The relationship between blood flow and oxygen consumption under conditions similar to those of these experiments is at present too inadequately determined (Barcroft and Muller, 1912; Verzar, 1912; Neuman, 1912; Nakamura, 1921; Yamakita, 1922) to permit conclusion as to whether oxygen consumption is secondarily related to vasoconstriction or both are independently affected. In any event these results confirm previous observations of Griffith and Hummel (1930) and in part, of v. Euler (1931); the latter's contention that minimal effective concentrations of adrenalin are dilator and increase oxygen consumption is, however, not substantiated; and if the vascular effect of adrenalin is of prime importance the subject is still further obscured by the report of Flatow and Morimoto (1928) that intra-arterially injected adrenalin is vasodilator in all concentrations except those in excess of anything used in this work or by the others just mentioned.

Previous reports on the effect of adrenalin on tissue carbon dioxide production (Martin and Armitstead, 1922; Garrey, 1922, Hutchinson, 1922; Griffith, 1923), even if in agreement, would probably be of small import in explanation of the apparently complex effect observed here. For reasons previously mentioned it would seem doubtful that a direct effect on cellular oxidative formation is at issue. Rather, a combined result of vasoconstriction and tissue glycogenolysis (Cori, 1931) seems suggested. Thus a concentration of adrenalin too small to increase vasoconstriction and without direct effect on oxygen utilization, might, provided a sufficiently lower glycogenolytic threshold and lactic acid formation, result in increased liberation and output of carbon dioxide. As vasoconstriction came increasingly into play with increasing dosage, liberation and output would be augmented less and finally even suppressed during injection itself, the output increasing only after injection is over and normal blood flow is resumed. Whether the proposed explanation is tenable or not this will be recognized as an exact description of the results observed.

The calorigenic action of adrenalin for the organism as a whole is incontestably established (Boothby and Sandiford, 1921 and 1923; Hunt and Bright, 1926); unpublished experiments of our own confirm this and show that under the same anesthesia and other conditions similar to those obtaining in this work, maximal increase in oxygen consumption and carbon dioxide output occur during a similar five-minute (intravenous) injection, or the ten minutes following. Therefore objection cannot be made that failure to observe an increase in the respiratory metabolism of the leg in this work is due to too short an experimental period. Rather,

the implication is strong that locus of the calorigenic effect is elsewhere than in the peripheral tissues.

SUMMARY

Intra-arterially injected adrenalin in any effective concentration reduces blood flow and oxygen consumption of the intact hind leg of the cat.

Carbon dioxide output is affected in a more complex manner suggesting a non-oxidative effect and removal dependent on the rate of effective blood flow.

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THE ABSORPTION OF PROTEIN SPLIT PRODUCTS FROM CHRONIC ISOLATED COLON LOOPS

JONATHAN E. RHOADS,[1] ALFRED STENGEL, JR.,[2] CECILIA RIEGEL, FLORIAN A. CAJORI AND WILLIAM D. FRAZIER

From the Harrison Department of Surgical Research, the Department of Physiological Chemistry, and the Department of Medicine, School of Medicine, University of Pennsylvania, Philadelphia

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There is now adequate evidence that a deficiency of the labile and reserve protein stores of the body is of serious consequence in a variety of conditions the surgeon is called upon to treat. Whipple (1938) has recently reviewed the subject of protein production and exchange in the body. Admont Clark (1919) called attention to the effect of a high protein diet on shortening the period of delay in wound healing. Thompson and his associates (1938) have demonstrated a relationship between wound healing and hypoproteinemia. The observations of Goldschmidt, Vars and Ravdin (1939) demonstrate the value of adequate protein in the diet, in the protection of liver from injury by volatile anesthetics. These observations in addition to those made in this laboratory (1937) on the effect of hypoproteinemia on gastric emptying have stimulated us to investigate methods by which the protein requirements of the patient may be met when the oral route is, for one reason or another, not available. This paper concerns itself with the problem of the absorption of protein from the large bowel of the dog. Studies which we had made in man suggested that this route of absorption was of greater value than it is generally supposed to be.

METHODS. A. *Loops of large intestine.* These were prepared in four dogs operated on under ether anesthesia, in two stages, three weeks apart. The first stage consisted of an ileo-sigmoidostomy. The second combined an appendicostomy with division of the small and large intestine, the former at the ileo-cecal junction and the latter just proximal to the ileo-sigmoidostomy. The loop so obtained was, therefore, closed at the sigmoid and open externally on the anterior wall at the cecum.

B. *Loops of small intestine.* One animal only of this type was used. The loop was prepared according to the technic described by Johnston (1932).

[1] John Berton Carnett Fellow in Surgical Research.

[2] Lyman Fellow in Medicine.

In both types of loop the technic of introducing the material to be studied and of retaining it in the loop for the desired length of time was that described by Johnston (1932). Before beginning each experimental period the loops were thoroughly washed out with physiological salt solution. Secretions from the colon loops have been studied for the presence of proteolytic enzymes. Incubation of a peptone solution with loop secretions failed to show any proteolytic action of the loop secretion.

Two types of material were studied: 1, a mixture of amino acids prepared by the acid hydrolysis of casein, the analysis of which by our methods gave approximately 80 per cent of the total nitrogen as amino nitrogen. This solution was diluted approximately 1:1 with 0.85 per cent sodium chloride for use in the loop. 2. A tryptic digest of Witte's peptone containing approximately 50 per cent of the total nitrogen as amino nitrogen. This solution was diluted approximately 1:1 with 0.85 per cent sodium chloride before use in the loop.

Fifty cubic centimeters of the material, the total nitrogen and amino nitrogen content of which were determined, were introduced into the colon loop, allowed to remain for approximately two hours and then removed by means of a syringe. In the small bowel loop the amount introduced was 35 cc. The loop was washed out two or three times with physiological saline solution, and the washings added to the material removed. Determinations of total nitrogen and amino nitrogen were made on these specimens. Total nitrogen was determined by the macro Kjeldahl method as described by Peters and Van Slyke (1932), and amino nitrogen by formol titration. Any protein material secreted into the loop during the course of the two hours was removed by preliminary treatment with heat and acetic acid.

Blank experiments were run to determine the amount of nitrogen in the secretion put out by the loop. Physiological saline was put into the loop, allowed to remain for two hours, then removed, and analysed for total nitrogen and amino nitrogen. Five such experiments were made on each dog. The average values so obtained were then subtracted from the figures obtained for total nitrogen and amino nitrogen in the material withdrawn after the experimental periods. Total nitrogen in the blanks was done by the micro Kjeldahl method as described by Peters and Van Slyke (1932).

Results. *Large intestine—Amino acid solution.* Eleven experiments were performed. The volume introduced, the concentration, and the time, remained constant. Fifty cubic centimeters of the diluted amino acid solution were introduced and for the eleven experiments (table 1) a mean of 72 cc. of solution was withdrawn from the loop two hours later. The mean disappearance of total nitrogen was 67 mgm. (21 per cent), while the mean disappearance of amino nitrogen was 57 mgm. (23 per cent).

Large intestine—Peptone hydrolysate. Twenty-two experiments were performed, divided into four groups (table 1) according to the amount of nitrogen introduced into the loop. A study of the sub-groups demonstrates that in general as the amount of total nitrogen was increased in the loops the rate of absorption increased. For example, in group A 257 mgm. (24 per cent) of the available 1089 mgm. of total nitrogen were absorbed, while in group D only 50 mgm. (25 per cent) of the available 201 mgm. of total nitrogen were absorbed. The percentage absorption remained fairly constant. Similar findings were obtained for the absorption of amino nitrogen, for here too, with increasing concentrations in the loops there was as a rule an increasing amount absorbed.

TABLE 1

SOLUTION	TIME	NO. OF EXPERIMENTS	VOLUME		AVERAGE AMOUNTS IN		AVERAGE CONCENTRATION IN		AVERAGE AMOUNTS OUT		AVERAGE AMOUNTS ABSORBED			
			In	Out	Total N	Amino N	Total N	Amino N	Total N	Amino N	Total N		Amino N	
Large intestine														
	hrs.		*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm. per cent*	*mgm. per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
Amino acid	2	11	50	72	316	251	732	502	249	194	67	21	57	23
Peptone hydrolysate														
A	2.2	2	50	151	1089	518	2178	1036	832		257	24		
B	2	9	50	70	654	320	1308	640	460	195	194	30	125	39
C	2.2	2	50	56	506	245	1012	490	308	157	198	39	88	36
D	2	9	50	52	201	114	402	228	151	69	50	25	45	39
Small intestine														
Amino acid	2	4	35	52	162	135	462	386	83	74	79	49	61	45
Peptone hydrolysate	2	5	35	67	127	67	363	190	74	22	53	42	45	67

Small intestine. There were nine experiments in this group, four with the amino acid solution, and five with the peptone hydrolysate. As would be expected, absorption of nitrogen from both types of solution was demonstrated. A mean of 79 mgm. of total nitrogen (49 per cent) and 61 mgm. of amino nitrogen (45 per cent) was absorbed from the amino acid solution, and 53 mgm. of total nitrogen (42 per cent) and 45 mgm. of amino nitrogen (67 per cent) from the peptone hydrolysate.

DISCUSSION. Quantitative studies on the absorption of protein or its degradation products from the colon were reported from this clinic in 1902 (Edsall and Miller), from Great Britain in 1906 (Boyd and Robertson) and from Germany in 1909 (Abderhalden, Frank and Schittenhelm).

Most of the studies made after 1900 showed that this method of absorption fulfilled only a small fraction of the caloric requirements of the individual, and that the nitrogen balance could not be maintained by rectal feeding. The work of Abderhalden, Frank and Schittenhelm (1909) differed from the others in that the enemata were carefully predigested. Although only a single patient was studied, the observations were continued over a period of thirty-two days, and nitrogen equilibrium was obtained.

In 1912 Folin and Denis performed a series of acute experiments on the large bowel of cats in which they showed substantial rises in the nonprotein nitrogen concentration of the blood after injection of urea, alanine, or glycine into a segment isolated by ligatures. A similar but smaller rise was obtained after injection of a 23 per cent solution of Witte's peptone. No conclusions were drawn from these data regarding the form in which the peptone was absorbed. As the solution was injected shortly after the large bowel was ligated some proteolytic enzymes from the small intestine may, of course, have been present. If such were the case the rise in nonprotein nitrogen after peptone injection into the colon may have been due to absorption of the amino acid formed by the action of the enzymes.

In 1913 Bywaters and Short reported on the absorption from the rectum of patients of peptonized milk and of milk predigested for 24 hours with pancreatic juice. Their conclusions were based on variations in urinary nitrogen excretion. They did not regard the evidence for the absorption of peptonized milk as satisfactory and doubted that protein was absorbed from the large bowel.

Numerous studies have been made on the absorption of various amino acids from isolated segments of the intestine and from the gastro-intestinal tract as a physiological unit. The data from these two types of experiments are often not in agreement. Wilson and Lewis (1929) in a study of the rate of absorption of different amino acids when fed to rats concluded that the absorption velocity was apparently unrelated to the absolute quantity of amino acids in the intestine. They demonstrated, however, a different rate of absorption of various amino acids when fed by mouth. It is important that no generalizations be made from their experiments as to what may happen when isolated segments of the intestinal tract are studied. The part played by the stomach and duodenum in making foodstuffs acceptable to the jejunum has been reported for the experimental animal by Ravdin, Johnston and Morrison (1933) and for man by Karr, Abbott, Hoffman and Miller (1939).

It is possible that the differences in absorption which were observed in the two solutions used in our experiments may be due to the presence

in the peptone hydrolysate of a higher proportion of the more readily absorbable amino acids. The data from group C of the peptone hydrolysate experiments and those of the amino acid group show that the absorption of amino nitrogen was more rapid from the peptone hydrolysate than from the amino acid mixture. The mean absorption in the former was 88 mgm. (36 per cent) while that in the latter, where a similar amount, 251 mgm., was originally introduced into the loop, was only 57 mgm. (23 per cent).

In the experiments performed on the small intestinal loop, there was, as would be expected, definite evidence of absorption and where the two are comparable absorption from the small intestinal loop was greater than from the large intestinal loop. For example, in group D of the peptone hydrolysate experiments on large intestinal loops the concentration of total nitrogen (402 mgm. per cent) and amino nitrogen (228 mgm. per cent) are of a similar order of magnitude to those used in the small intestinal loop (363 mgm. per cent total nitrogen and 190 mgm. per cent amino nitrogen). If the rate of absorption were the same in the two loops one would expect a greater amount to be absorbed from the large intestinal loop since 50 cc. were introduced as compared with 35 into the small bowel loop. However, the amount of nitrogen absorbed in both groups was the same, indicating a greater rate of absorption from the 35 cc. in the small bowel as compared with the 50 cc. in the large bowel.

The data in groups B and C indicate absorption of protein split products in a less degraded form than the amino acids from chronic colon loops, the secretion from which did not cause further protein hydrolysis.

Our experiments indicate that it may be possible to supply the protein requirements of man by the colonic administration of a protein hydrolysate providing carbohydrates are simultaneously supplied by vein to decrease the protein requirements to a minimum.

SUMMARY

1. Amino acids are readily absorbed from chronic isolated loops of the large bowel in the dog.

2. There is evidence that some of the higher split products of protein digestion are absorbed from such loops.

3. Increasing concentrations of total nitrogen and amino nitrogen in the large bowel loop resulted in increasing amounts absorbed in a given time.

4. The rate of absorption of both types of substances is slower from the large than from the small bowel.

5. The rate of absorption of amino nitrogen was more rapid from the protein hydrolysate than from the amino acid mixture.

6. The importance of distinguishing results obtained from experiments in which the gastro-intestinal tract is used as a physiological unit from those in which only a segment has been used has been pointed out.

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THE EFFECT OF ADRENALINE, NEMBUTAL AND SYMPATHECTOMY ON THE PLASMA VOLUME OF THE CAT[1]

EDWARD HAMLIN AND MAGNUS I. GREGERSEN

From the Departments of Physiology of the University of Maryland School of Medicine and the Harvard Medical School, and the Surgical Services of the Massachusetts General Hospital

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It has been reported (Bainbridge and Trevan, 1917; Erlanger and Gasser, 1919; Freeman, 1933; and others) that the continuous intravenous injection of adrenaline causes a considerable loss of fluid from the circulation. Freeman (1933) states that this also results from hyperactivity of the sympathetic system and considers it an important factor in the production of surgical shock. That it represents the normal effect of adrenaline and the sympathetic system on plasma volume is, however, open to some question since the evidence has been obtained largely from studies on anesthetized animals. The purpose of the present investigation was to study the influence of the sympathetic system upon the volume of circulating plasma without employing anesthetics.

METHODS. Adult male cats were used throughout. A method was devised for tying the unanesthetized cat to an animal board without obstructing the circulation through the extremities. The principal restraints consisted of a broad canvas belt covering the body from axilla to groin, and a bar which prevented the cat from raising its head. The leg thongs were tied as loosely as possible. The animals became accustomed to this procedure surprisingly soon and after two or three trials they seldom attempted to struggle during the course of an experiment. The average heart rate of cats tied down in this manner was 140 beats per minute. In normal unanesthetized cats permitted to rest in a position of their own choice, Moore and Cannon (1930) found that the minimum heart rate was about 95 beats per minute.

Plasma volume determinations were made with the blue dye T-1824 (Gregersen et al., 1935, 1937; Gregersen, 1938a, 1938b). A sufficient amount of dye (usually 0.3 cc. of a 1 per cent solution) was injected in order to make possible an accurate spectrophotometric analysis on less than 0.5 cc. of serum. The saphenous veins were used for the injections

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of dye and the taking of blood samples. Any changes in plasma volume that occurred as a result of injection of adrenaline or nembutal were calculated from the deviation of the disappearance curve (see figs. 2 and 3). Re-injection of dye at the conclusion of about half of the experiments showed that the changes estimated from the first disappearance curve were essentially correct. Additional evidence of alterations in the plasma volume was obtained from plasma protein determinations (Abbé refractometer) and occasionally from hematocrit readings.

Adrenaline (1:1000 adrenalin, Park, Davis) was injected from a calibrated syringe into the saphenous vein which was not being used for the taking of blood samples. The plunger of the syringe was driven by an electric motor through a set of reduction gears, affording a constant rate of injection. The total amount of solution injected never exceeded 3 cc., and in most of the experiments it was less than 1 cc. The doses of adrenaline ranged from 0.035 mgm. to 0.0018 mgm. per kgm. per min., the average for all experiments being 0.0142 mgm. per kgm. per min. It should be noted that this exceeds by about four times the rate at which adrenaline is secreted in response to afferent stimulation (0.0035–0.0037 mgm. per kgm. per min.) (Cannon and Rapport, 1921). Nembutal,[2] when used, was injected intravenously in the usual anesthetic dose (0.5–0.7 cc. per kgm.).

Results. *Plasma volume of normal unanesthetized cats.* Seventy-three determinations were made on 52 animals in which the body-weights ranged from 1.95 to 5.4 kgm. The plasma volumes, expressed in cubic centimeters per kilogram, ranged from 34 to 56. Nevertheless, more than one-half of the values fell within ±3 cc. of the average 47.7 cc. per kgm. The variations do not appear to be related to differences in body weight.

Effect of sympathectomy. The plasma volume was determined before and after complete sympathectomy in 8 cats (table 1). Four of these, 1, 4, 5 and 7, showed a remarkable increase in plasma volume, the greatest change occurring in cat 4 with an average increase of 55 per cent. It will be seen that definite changes occurred also in 2, 3 and 8. The average increase in these 7 animals was 26.2 per cent. The estimates were made by comparing the highest pre-operative plasma volume in cubic centimeters per kilogram with the average of the post-operative determinations.

In one cat, no. 6, the plasma volume showed no change, but it should be noted that in this case the post-operative determinations were not made until 5 months after the first stage of sympathectomy during which time regeneration of preganglionic fibers to prevertebral ganglia may have

[2] Supplied by Abbott Labotatories.

TABLE 1

The effect of total sympathectomy on the plasma volume

CAT NUMBER	DATE	BODY WEIGHT	PLASMA VOLUME	CHANGE	COMMENTS
		kgm.	*cc. per kgm.*	*per cent*	
1	9/21/36	3.6	45.5		Pre-operative
	11/13/36				Operation complete
	11/18/36	2.6	55.5	22.0	
	11/23/36	2.8	59.5	31.0	
	11/27/36	2.7	55.0	21.0	
2	9/ 2/36	3.8	45.0		Pre-operative
	9/ 9/36				1st stage complete
	10/ 2/36				Operation complete
	11/ 2/36	3.7	44.0	−2.2	
	11/ 4/36	3.65	49.3	9.6	
	11/10/36	4.0	48.0	6.7	
	11/12/36	4.0	49.0	8.9	
	11/30/36	4.0	49.0	8.9	Very slight dilution after i.v. nembutal
3	9/10/36	3.0	50.5		Pre-operative
	9/17/36				1st stage operation
	10/ 5/36				Operation complete
	11/ 5/36	2.7	59.0	16.9	
4	5/25/36	3.5	36.0		Pre-operative
	6/ 8/36	3.3	39.0		Pre-operative
	6/ 9/36				1st stage operation
	6/21/36				Operation complete
	6/28/36	2.85	55.0	41.0	
	7/22/36	3.3	65.0	67.0	
	7/31/36	3.7	62.0	59.0	
	8/ 4/36	3.4	59.0	51.0	
5	5/28/36	3.75	39.0		Pre-operative
	6/ 4/36	3.5	42.0		Pre-operative
	6/ 5/36				1st stage operation
	6/21/36				Operation complete
	6/28/36	2.35	62.0	47.5	
6	4/ 6/37	3.2	48.5		Pre-operative
	4/27/37	3.0	48.5		Pre-operative
	6/14/37	4.05	45.5		Pre-operative
	7/22/37				1st stage operation
	11/11/37				Operation complete
	12/11/37	3.8	47.8	3.9	5 months post-operative
	1/21/38	4.25	44.3	−3.7	6.4 per cent dilution after nembutal
7	6/28/37	3.35	32.0		Pre-operative
	7/12/37	3.35	36.0		Pre-operative
	8/17/37	3.1	41.0		Pre-operative
	9/22/37				½ sympathectomized
	10/ 8/37	3.07	40.5		
	11/21/37				Operation complete
	12/13/37	3.25	50.0	22.0	
8	7/27/37	3.3	53.0		Pre-operative
	9/ 1/37	3.8	54.0		Pre-operative
	11/ 6/37				¾ sympathectomized
	11/18/37	2.8	59.0	9.5	

occurred. That this cat differed from recently sympathectomized animals is also revealed by the intravenous injection of nembutal which in cat 6 caused a 6 per cent increase in plasma volume, whereas in cats 1 and 2, the effect of nembutal on plasma volume was slight (see table 3).

Two incidental observations deserve mention. The animals were always deprived of food for twenty-four hours prior to the determination of plasma volume. Under these conditions the serum samples from normal cats were invariably clear, but in the sympathectomized cats there was such a high incidence of lipemia that it occasionally interfered with the determinations. Another difference was observed in the rate of disappearance of T-1824 from the plasma. The slope of the disappearance curve was approximately one-half as great in the sympathectomized as in the normal cats.

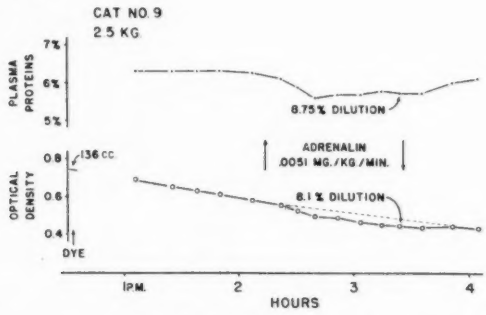

Fig. 1. Normal unanesthetized cat. Showing the dilution of plasma proteins and dye during the intravenous injection of adrenaline at the rate of 0.0051 mgm. per kilo per minute.

Effect of continuous injection of adrenaline. The results of 11 experiments on 10 normal unanesthetized cats are presented in table 2. In 8 of these the plasma volume increased from 2 to 11 per cent, one (no. 12) showed no change, and in two there was a decrease of about 3 per cent. In these two experiments the animals showed marked cardiac irregularity during the injection. Furthermore, a repetition of the experiment on cat 16, given somewhat less adrenaline, produced an increase in plasma volume. It may be seen from figure 1 that dilution of the plasma occurred within a few minutes after the injection was started and that no further change took place during the remainder of the infusion. Certainly there was no evidence of a progressive reduction in plasma volume even in the experiments in which the injection was continued for more than one hour. The results of tests carried out on 4 sympathectomized cats are also included in table 2. Here again adrenaline caused dilution of the circulating dye.

The method employed above for estimating changes in plasma volume during adrenaline injections does not of course exclude the possibility of a reduction in the volume of circulating plasma as a result of pooling of blood in some parts of the vascular bed which may have been cut off from active circulation by arteriolar constriction. This possibility was investigated in two experiments by injecting the dye after the infusion of

TABLE 2

The effect of continuous intravenous injections of adrenaline on the plasma volume of unanesthetized cats

CAT NUMBER	DATE	BODY WEIGHT	PLASMA VOLUME			ADRENALINE		COMMENTS
			Normal	During adrenaline	Change	Rate of injection	Duration	
			A. Normal cats					
		kgm.	*cc.*	*cc.*	*per cent*	*mgm./kgm./min.*	*min.*	
4	5/25/36	3.3	112	118	+5.3	0.025	27	
9	7/21/36	2.5	136	147	+8.1	0.0051	72	
10	5/20/36	2.5	120	129	+7.5	0.0127	22	
11	5/19/36	2.9	146	149	+2.0	0.0136	20	
12	5/22/36	2.9	117	118		0.035	24	
13	5/15/36	3	151	168	+11.0	0.018	20	
14	6/ 1/36	2.95	125	121	−3.2	0.0057	45	Marked cardiac irregularity
15	5/13/36	3.65	139	142	+2.1	0.005	12	
16	5/29/36	3.3	144	140	−2.8	0.014	34	Heart very irregular. Pulse varied 90-240 per minute
16	6/13/36	3.3	145	149	+2.7	0.0067	31	
17	6/28/36	3.75	146	151	+3.4	0.0055	40	
			B. Sympathectomized cats					
2	11/ 2/36	3.7	164	170	+3.6	0.0045	58	Postoperative 6 weeks
1	11/23/36	2.8	165	173	+4.8	0.0057	39	4 weeks
4	7/22/36	3.3	216	245	+13.5	0.0047	63	6 weeks
3	11/ 5/36	2.7	160	163	+1.0	0.0047	75	6 weeks

adrenaline had been started. Under these conditions the dye would be diluted only by plasma in active circulation and the reëntry of dye-free plasma, pooled during the infusion, should be apparent from the disappearance curve. In both experiments the plasma volume was greater during the administration of adrenaline than subsequently.

Effect of adrenaline on the plasma volume in cats anesthetized with nem-

butal. The experiments were carried out on 6 normal and 3 sympathectomized cats. It will be seen from the data presented in table 3 and figure 2 that the intravenous injection of nembutal in normal cats causes an immediate increase in plasma volume of about 10 per cent. The same result was obtained in one experiment in which dial was given by stomach tube. Adrenaline, injected shortly after nembutal, produced a relative decrease in plasma volume, an effect opposite to that obtained in the unanesthetized animal. This decrease averaged 9 per cent and in no case did it exceed the increase produced by the anesthetic (see table 3

TABLE 3

Showing the changes in plasma volume produced by the intravenous injection of nembutal (anesthetic dose) and the subsequent intravenous injection of adrenaline

CAT NUMBER	DATE	BODY WEIGHT	PLASMA VOLUME					ADRENALINE		COMMENTS
			Initial	After nembutal	Change	During adrenaline	Change	Rate of injection	Duration	
A. Normal cats										
		kgm.	*cc.*	*cc.*	*per cent*	*cc.*	*per cent*	*mgm./kgm./min.*	*min.*	
18	6/15/36	2.9	126	137	+8.7	128	−6.5	0.023	26	
19	6/11/36	2.1	108	125	+15.0	108	−13.5	0.0078	24	
20	6/13/36	3.5	161	185	+15.0	161	−13.0	0.0176	26	
21	6/17/36	2.8	138	150	+8.7	142	−5.3	0.024	25	
22	6/15/36	3.5	175	190	+8.6	175	−7.9	0.0217	26	
23	6/13/36	3.4	151	161	+6.1	155	−3.7	0.0044	20	
B. Sympathectomized cats										
1	11/27/36	2.7	147	150	+2.0	133	−11.3	0.0067	40	Postoperative 3 wks.
2	11/12/36	4	197	201	+2.0	177	−12.0	0.0033	61	4 wks
2	11/30/36	4	194	195	+0.5	178	−8.7	0.0051	39	7 wks
6	1/21/38	4.2	188	200	+6.4					5 mos.

and fig. 2). In sympathectomized cats (table 3) nembutal caused an increase in plasma volume of only 2 per cent, except in cat 6, in which regeneration of splanchnic connections may have occurred. A subsequent injection of adrenaline, however, resulted in a decrease of about 11 per cent, i.e., 9 per cent below the pre-anesthetic level.

Effect of hyperactivity of the sympathetic nervous system on plasma volume. A number of cats were decorticated under ether anesthesia in an effort to produce pseudaffective preparations. For the most part these were unsatisfactory, but in 7 animals, which displayed the typical picture of "sham rage," a decrease in plasma volume was observed only once and

that in a cat in which the respiratory rate was 280 per minute for 90 minutes. Further study of the pseudaffective preparations did not seem worth while since the effect of sympathetic stimulation cannot in these experiments be separated from that produced by struggling. In man, Gregersen, Dill and Meade (Gregersen, 1938a) have found that a short bout of violent muscular activity causes a striking reduction in plasma volume, and it has been observed frequently by the present authors that if an animal struggles during the determination of plasma volume there is an increase in the plasma dye concentration associated with the periods of struggle.

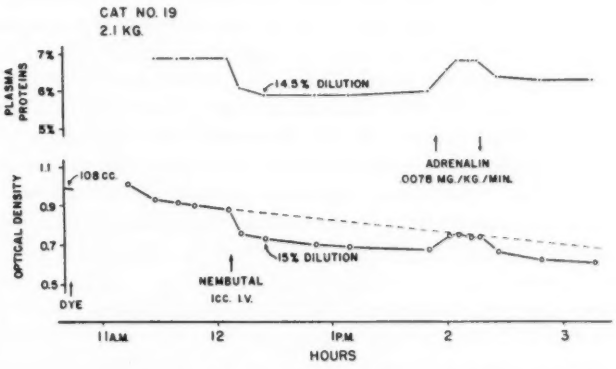

Fig. 2. Normal cat. Showing the changes in the concentration of plasma proteins and dye following the administration of nembutal and during the subsequent infusion of adrenaline (0.0078 mgm. per kilo per min.).

Discussion. In normal cats the average plasma volume per kilogram body weight was found to be about 15 per cent higher than the values reported by Harris (1920) and by Freeman (1933). The difference is probably attributable to differences in the methods employed for determining plasma volume. Both Harris and Freeman based their calculations on the dye concentration in plasma samples obtained about 5 minutes after the injection. According to our time-concentration curves, it usually takes about 20 minutes for the dye to become uniformly distributed in the circulation of normal cats. Hence, 5 minutes after injection the dye-concentration yields a low value for plasma volume.

Our observations on the effects of adrenaline on plasma volume appear to be at variance with those of Freeman (1933) and others. It should be noted, however, that Gregersen and Pinkston (1936) also found evidence of an increase in plasma volume in a considerable proportion of their experiments on unanesthetized dogs during the infusion of adrenaline.

The investigators who have reported that a condition resembling "shock" results from prolonged injections of adrenaline performed their experiments under anesthesia. That this plays an important rôle in determining the effect of adrenaline is clearly shown in the evidence presented above. In normal cats the reduction in plasma volume appeared only when the adrenaline injections were preceded by the administration of nembutal and even then the change was hardly sufficient to counteract the dilution caused by the anesthetic. Furthermore, Prohaska, Harms and Dragstedt (1937) state that in unanesthetized dogs kept in a state of hypertension for as long as two weeks by the continual injection of adrenaline, the most probable cause of death was inhibition of gastrointestinal motility and derangement of the carbohydrate metabolism.

The changes in plasma volume produced by adrenaline or nembutal must in the final analysis be attributed to disturbances in either capillary pressure or permeability or to both. That either of these substances alone should cause dilution of the plasma in the normal cat is rather striking since adrenaline produces generalized vasoconstriction whereas a barbiturate, e.g., nembutal, produces vasodilatation (Novak, 1934; Tournade and Joltiain, 1935; Hausner, Essex and Mann, 1938) and vascular engorgement (Carriere, Heriez and Willoquet, 1934). Equally puzzling are the observations on sympathectomized cats. In these as in the normal cats, adrenaline alone caused an increase in plasma volume, whereas nembutal failed to do so to any significant extent. Nevertheless, after the injection of nembutal, adrenaline reduced the plasma volume.

Several workers have studied the effect of sympathectomy on blood pressure. In dogs Bradford Cannon (1931) found that the blood pressure was reduced only slightly after total extirpation of the sympathetic chains and this has been confirmed by Wilson, Roome and Grimson (1936). From the fact that the bleeding volume was not increased, the latter authors inferred that there was no change in plasma volume. Hemorrhage has long been known to cause vaso-constriction in normal animals (Pilcher and Sollmann, 1914). It seems reasonable to suppose that in sympathectomized animals, in which there can be no reflex vaso-constriction, the hemorrhage required to lower the blood pressure to any given point will be proportionately less than in a normal animal. Therefore, if the bleeding volume of normal and sympathectomized animals is of the same magnitude, the sympathectomized animal must have a larger blood volume than the normal. The evidence here presented shows that such is the case.

The recovery of the blood pressure to normal after partial or total sympathectomy cannot be wholly explained by the gradual establishment of intrinsic vascular tone, for recent work (Herrick, Essex and Baldes, 1933) demonstrates that the blood flow in sympathectomized limbs re-

mains permanently increased. The present study suggests that restoration of the blood pressure is brought about by an increase in the plasma volume commensurate with the increased capacity of the circulatory system resulting from section of the vasomotor nerves.

SUMMARY

1. In 73 determinations on normal unanesthetized cats the average plasma volume was 47.7 cc. per kgm. body weight.

2. In unanesthetized normal and in sympathectomized cats, the continuous intravenous injection of adrenaline (0.0018 to 0.035 mgm. per kgm. per min.) caused an increase in plasma volume (table 2).

3. The intravenous injection of nembutal causes an increase in plasma volume in normal cats of 10 per cent but an increase of only 2 per cent or less in sympathectomized cats.

4. Both normal and sympathectomized cats anesthetized with nembutal show a decrease in plasma volume when adrenaline is injected. The plasma volume does not, however, fall below the pre-anesthetic level in normal cats (table 3).

5. In 7 out of 8 completely sympathectomized cats the average plasma volume was 26 per cent higher than before the operation (table 1).

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THE RACIAL FACTOR IN THE PIGEON CROP-SAC METHOD OF BIOASSAY OF PROLACTIN

ROBERT W. BATES, OSCAR RIDDLE AND ERNEST L. LAHR

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

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We have indicated in published abstracts (1, 2) that races (and strains) of doves and pigeons differ markedly in the extent of their crop-sac (weight) response to prolactin. This paper presents our data on this racial factor. The results of this study require rather full consideration because they direct attention to a little known aspect of genetic difference and because the crop-sac is now widely used in the quantitative assay of prolactin. Racial variation in the crop-sac response is of such magnitude that only inbred strains of pigeons may be expected to give reproducible values for assay purposes.

Although the fact is widely recognized there has been surprisingly little published concerning the relative responsiveness in bioassays of different races or strains of experimental animals. Besides the present cases, already reported in abstract, we can cite only four others. Acton and Bose (3) found that under injection of insulin or adrenalin the change in blood sugar level differed significantly in three breeds or races of Himalayan rabbits; these races had been derived in the process of segregating three color types. Cole and Harned (4) found two strains of rats in which glucose tolerance differed markedly, with one of the strains giving a diabetic curve. Rotter and Mecz (5) reported that albino guinea pigs are more sensitive to thyroid preparations than are pigmented guinea pigs. Very recently Light and Cracas (6) noted that in order to obtain equal growth responses one strain of rats (in their own colony) required twice as much vitamin B_1 as another strain.

Perhaps racial difference in responsiveness of tissues—if responsiveness of tissue proves to be the essential factor in the above-named cases—is involved in many genetic differences. The unequal milk-producing abilities of well-known breeds of cattle and the dissimilar egg-laying abilities of various races of fowl are recognized racial variables; possibly these differences reflect an inherently different endocrine status (or differing responsiveness of tissues) along with other aspects of genetic difference. Riddle, Bates and Lahr (7) found that injections of prolactin into laying

fowl produces broodiness and noted that hens from genetically non-broody races showed a marked quantitative difference in response (clucking only) from that of hens of "broody" races. The fact that a genetic basis exists for this difference in response of fowl to prolactin may have bearing upon an interpretation of the differences in response here observed on the crop-sacs of pigeons.

EXPERIMENTAL ANIMALS. The races of doves used in our tests were inbred races (or hybrids from two such races) established 14 to 17 years ago (by O. R.) upon a basis of heritable differences in thyroid weight or intestinal length. All these dove races have essentially the same body weight (150 grams) and unstimulated crop-sac weight (<400 mgm.). The pigeon races used are likewise those that have been maintained in our colony for over 15 years except for three commercial strains called Carneaux, Homers and White Kings. The pigeon races differ much in body weight (280–600 grams). Because of the narrow age limit (6 to 10 weeks after hatching) of birds suitable for these tests and because of the small number of available birds of each race in our colony we have been limited to a small number of birds in each racial group. The differences found, however, are of such magnitude that the results have significance despite the small numbers used in the various groups.

METHOD OF INJECTION AND CALCULATION. All birds were injected once daily for four days and killed 24 hours after the last injection (96 hours after first injection). All injections were alternated daily from right to left side of the breast. Intramuscular injections were made deep into the pectoral muscles and subcutaneous injections were made under the skin of the breast region. Prolactin preparation no. 296, containing 4 units per milligram, was used throughout. The injection procedures used here were identical (intramuscular series) with those used by us for assaying prolactin and therefore we can convert crop-sac weights directly into equivalent unitage of prolactin by means of our assay table published elsewhere (8). Since the practical importance of the racial difference lies in its effect on assay values our results for the various races have been so tabulated as to give comparisons in terms of units as well as in terms of crop-sac weights.

EXPERIMENTAL RESULTS. *Intramuscular injection in doves.* Tables 1 and 2 show crop-sac weights, and prolactin unitages corresponding to those weights, obtained from several ring dove races or strains following intramuscular injections of prolactin at *two* dosage levels. The races are arranged in the order of their responsiveness—as this was indicated by the crop-sac weights obtained. With the notable exception of race *W* this sequence (for those races which were treated at both levels) is essentially the same at both levels of dosage. There is little or no overlap between the data obtained from the races at the extreme top and those at the bottom

of the tables. The maximum racial difference in response, in terms of units, is a 24-fold difference under high dosage (table 1) and an 8-fold difference under low dosage (table 2). If unrecognized as such these racial differences would greatly confuse or invalidate a bioassay.

TABLE 1

Differences in response to high dosage of prolactin in races of ring doves. Ten milligrams (40 units) of no. 296 injected intramuscularly daily for 4 days

RACE	NO. OF BIRDS	CROP-SAC WEIGHT				RESPONSE OBTAINED	RATIO TO 160 UNITS
		Minimum	Maximum	Average	σ_{mean}		
		mgm.	*mgm.*	*mgm.*		*units*	
63x44	5	2,010	2,505	2,320	±91	360	2.2
N2x62	9	1,630	2,475	2,060	±75	165	1.0
72	7	1,650	2,470	1,880	±108	94	0.59
63	14	1,270	2,185	1,810	±108	75	0.47
62	6	1,410	2,055	1,800	±87	74	0.46
NAx72	7	1,195	2,215	1,590	±130	39	0.24
63x72	7	1,290	1,895	1,580	±82	37	0.23
62x75	8	1,060	2,315	1,560	±144	35	0.22
51x63	6	1,230	1,755	1,540	±87	33	0.21
51	5	1,290	1,935	1,440	±123	34	0.15
W	8	925	1,655	1,270	±72	15	0.09

TABLE 2

Differences in response to low dosage of prolactin in races of ring doves. Four-tenths milligram (1.6 units) of no. 296 injected intramuscularly daily for 4 days

RACE	NO. OF BIRDS	CROP-SAC WEIGHT				RESPONSE OBTAINED	RATIO TO 6.4 UNITS
		Minimum	Maximum	Average	σ_{mean}		
		mgm.	*mgm.*	*mgm.*		*units*	
63x44	5	1,095	2,025	1,440	±161	24	3.8
N2x62	5	955	1,495	1,310	±105	14	2.2
63x0	5	865	1,445	1,195	±102	11.5	1.8
Bx62	7	645	1,945	1,090	±183	8.4	1.3
62x63	5	945	1,165	1,085	±49	8.3	1.3
63x72	5	765	1,385	1,000	±104	6.3	0.98
W	7	705	1,180	970	±70	5.7	0.89
72	6	650	1,160	945	±73	5.3	0.83
63	5	670	1,425	945	±128	5.3	0.83
NAx72	5	730	1,030	865	±56	4.2	0.66
51x63	5	520	1,070	845	±100	3.9	0.61
72x75xB	5	605	1,150	840	±103	3.8	0.59
65	5	665	905	800	±43	3.4	0.53
72x0xNA	5	500	1,020	740	±98	2.9	0.45

Most of the data in table 1 were obtained in 1934 while those in table 2

were obtained since 1935. On the basis of the data of table 1 races N2x62 and *W* were selected in 1935 as representative of high-responding and low-responding races and their response with variable prolactin dosage was investigated with the results shown in figure 1.

When crop-sac weights are plotted against the logarithm of the prolactin dosage (fig. 1) the experimental points fall along a straight line of characteristic slope—this slope being independent of age and, after correction for differences in body weight, also quite the same for pigeons as for ring doves All the points for race N2x62 (fig. 1), with the exception of the one for the

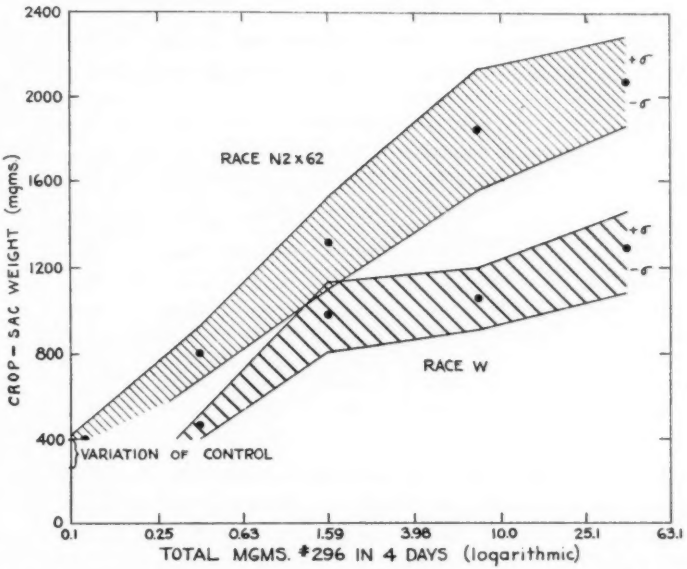

Fig. 1. Response of two ring dove races to prolactin. Points represent average weights of crop-sacs from 4–9 doves.

highest dosage, fall along a line with the characteristic slope. In the case of race *W* only the points for the two lowest dosages (0.4 and 1.6 mgm.) fall along a line with the usual slope; increasing the dosage 5 times (to 8 mgm.) did not here appreciably increase the crop-sac weight, and increasing the dosage by 25 times (to 40 mgm.) did little more to the crop-sac weight than would be expected from an increase of dosage to 4 mgm. At the two low levels of dosage race *W* required 2.5 times more prolactin than did race N2x62 to produce equal crop-sac weights. Obviously race *W* is of no value for quantitative assay of prolactin unless one works at low dosage levels of only four to five times the minimal stimulating dose for

race *W*. Race N2x62 can be used for assaying at dosage levels up to 50–100 times the minimal stimulating dose for N2x62—a value which is 20–40 times the minimal stimulating dose for race W.

Referring again to the data in tables 1 and 2 it was found that only races 63 and 72 gave crop-sac weight differences at the two levels of dosage which, when converted into units, approached a 25-fold difference in dosage—an 18-fold difference was found here. This poor showing for the method of calculation is due to the unfortunately high dosage level used for our first series of experiments (table 1). This dosage level (160 units) is above the useful range of response for *most* races of doves; this fact was learned only in our subsequent studies.

Subcutaneous injection in doves. Bates and Riddle (9) found that in Carneau pigeons prolactin injected subcutaneously is four times as effective

TABLE 3

Differences in response to low dosage of prolactin in races of ring doves. Four-tenths milligram (1.6 units) of no. 296 injected subcutaneously daily for 4 days

RACE	NO. OF BIRDS	CROP-SAC WEIGHT				RESPONSE OBTAINED	RATIO TO 6.4 UNITS
		Minimum	Maximum	Average	σ_{mean}		
		mgm.	*mgm.*	*mgm.*		*units*	
72	5	1,010	1,590	1,200	±105	11.7	1.8
63x72	5	970	1,560	1,190	±105	11.5	1.8
NAx72	5	710	1,450	1,150	±138	10.0	1.56
Bx62	5	510	1,360	940	±151	5.4	0.85
65	5	600	1,260	865	±106	4.1	0.64

as intramuscular injection. It was therefore useful to learn whether this difference applies to ring doves and also to learn whether racial differences are exhibited after subcutaneous injections of prolactin. Table 3 shows results obtained on five races of doves. The maximum increase in effectiveness (for any dove race) of subcutaneous injection over intramuscular injection was only two-fold. One race (Bx62) seems to show a slight decrease rather than an increase, but this indication is not really significant since the race is a highly variable one. The available data suggest that racial differences under subcutaneous injection are about the same as those obtained under intramuscular injection.

Intramuscular injection in pigeons. Since most workers with prolactin have only pigeons available for assay purposes we have tested several races of pigeons at high and low levels of prolactin dosage—the higher dosage being 25 times the lower as in the case of doves. This dosage, however, was relatively less than for the doves due to the greater body weight of the pigeons. Table 4 presents the data. Because of the variation of body weight among pigeon races the crop-sac weights have been

calculated to equivalent body weights (450 grams). The birds were injected with amounts of prolactin in proportion to their body weight. A body weight of 450 grams was made the basis of calculation because that value is nearly the average body weight of young Carneau pigeons and because it is exactly three times the body weight of ring doves. The values could have been calculated equally well to a body weight base of 150 grams.

A maximum racial difference of more than 12-fold was found to exist among the six pigeon races tested. Three of the four races tested at both levels of dosage show differences in response remarkably near the expected difference. White Kings, however, were found to be in the region of their

TABLE 4

Differences in response to two dosage levels of prolactin in six races of pigeons. Low dosage (0.5 mgm. or 2 units) and high dosage (12.5 mgm. or 50 units) of no. 296 injected intramuscularly daily for 4 days. Actual dosage was proportional to body weight.

RACE	NO. OF BIRDS	BODY WEIGHT	TOTAL UNITS / 450 GRAMS	CROP-SAC WT./450 GRAMS				RESPONSE OBTAINED	RATIO TO 200 OR TO 8 UNITS
				Minimum	Maximum	Average	σ mean		
		grams		*mgm.*	*mgm.*	*mgm.*		*units*	
Homer	6	358	200	4,500	8,410	5,970	±543	395	2.0
	5	357	8	1,980	3,870	2,910	±361	17.3	2.2
White King	9	598	200	4,200	7,050	5,800	±378	330	1.7
	5	556	8	3,180	4,500	3,890	±231	47.2	5.9
Carneau*	5	439	8	1,500	3,360	2,520	±390	11.5	1.4
Homer*	5	394	8	1,625	2,280	2,070	±120	7.3	0.91
Tx267	12	309	200	3,150	5,130	4,470	±238	85	0.43
	5	318	8	1,125	1,905	1,545	±141	4.3	0.54
Tippler	9	292	200	3,660	5,550	4,490	±183	87	0.44
	5	263	8	1,155	1,640	1,410	±111	3.7	0.46

* Birds purchased from Palmetto Pigeon Plant, Sumter, S. C.

maximum response on the low dosage level; and only because they were the most responsive race of pigeons tested do they still show the high relative position in the table at the high level of dosage. Like race *W* among the doves our White Kings are not suitable for assay purposes by the weight method.

DISCUSSION. How is this difference in response that we measure by crop-sac weight to be explained? At least three possibilities must be considered. The difference may rest solely upon an inherent difference in the responding (crop-sac) tissue; in this case a hitherto practically unrecognized and important type of genetic difference is involved. The

hereditary difference may relate solely to the factors or mechanisms that determine the concentration and persistence in the blood stream of the injected hormone; such mechanisms, particularly those controlling the rates of absorption and elimination, may be considered as general metabolic mechanisms. A third possibility is that the observed difference rests upon both the responding tissue and upon such metabolic mechanisms.

Certain variations in the growth response (a response largely under pituitary control) seem to show different thresholds of response in the different tissues of the body. For example, it is well known that in most cases of acromegaly not all the tissues capable of responding actually do so. In relatively few cases do the viscera increase in size while the jaws show overgrowth most readily. Of very special interest to the question raised in this paper, however, are the cases cited by Lichtwitz (10) in which the right jaw but not the left showed overgrowth. Here apparently is a true difference in tissue responsiveness, and this type of (bilateral) difference may readily have a genetic basis—as have other better analyzed characteristics which appear on only a single side of the body.

The data presented clearly demonstrate significant differences in the crop-sac weights of various races or strains of doves and pigeons parenterally injected with equal amounts of prolactin. The maximum variation found in the races studied indicates that some races are more than 8 times as responsive as others. For any race of pigeons used in the quantitative assay of prolactin it is necessary to determine the useful range over which crop-sac weight increases with dosage and also to establish the approximate racial correction factor. Both of these things may be accomplished simultaneously by standardizing the race against a standard prolactin preparation. The ratios in the last column of tables 1 to 4 are such racial correction factors. Data obtained with larger numbers of Carneaux, and using other prolactin preparations than no. 296, indicate their racial factor to be *1.2*. It is because our prolactin unit was based on crop-sac data obtained from groups of doves belonging to several races (selected at random) that the data from the dove races of table 2 fall both above and below the theoretical value. There is no apparent correlation between gut length or thyroid weight and crop-sac response.

SUMMARY

Different races and strains of doves and pigeons may differ markedly in the response (increased weight) of their crop-sacs to prolactin when this hormone is injected either intramuscularly or subcutaneously. When the crop-sac responses obtained are expressed in terms of prolactin units an extreme variation of five-fold was found among six races of pigeons and of eight-fold among fourteen strains of doves. These variations were still more extreme when high dosage of prolactin was employed.

When any race of pigeons is used for quantitative assay of prolactin by the weight method it is first necessary to determine the useful range over which crop-sac weight increases with dosage, and thereafter to establish a racial correction factor with the aid of a standard prolactin preparation. The question of the source of differences in response of races is discussed.

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EMOTIONAL HYPERGLYCEMIA AND HYPERTHERMIA IN TROPICAL MAMMALS AND REPTILES

S. W. BRITTON[1] AND R. F. KLINE

From the Physiological Laboratory of the University of Virginia Medical School

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Contrasting extremes in activity which are shown by different mammals and reptiles in the American tropics present a stimulating physiological challenge as well as an interesting nature study. Monkeys and sloths are remarkably opposed examples, and all degrees of activity are observed between these forms. A relentlessly uniform thermal climate the year round is nevertheless characteristic of their habitat.

A common condition, emotion, and correlated changes in blood glucose and body temperature, have recently been studied in both the above active and relatively inactive animals forms, and comparison has been made with responses in a number of other tropical species. The possible detection of an evolutionary trend in psychosomatic expressions was considered. A large number of mammals and several reptiles were studied.

METHODS. The work was carried out under fairly ideal physiological conditions in a jungle laboratory or in a nearby station in Panama.[2] Freshly collected animals were used. Most of the experiments were run in the morning after the animals had fasted approximately 12 hours over night. Normal or preëxcitement blood samples were taken with the least disturbance of the animal and as quickly as possible after withdrawing it from the cage. Blood glucose determinations were made according to the method of Folin and Malmros (1929). Deep rectal temperatures were taken.

Emotional excitement was readily elicited in nearly all specimens. Usually an animal was restricted or cornered in a cage or some part of the laboratory by one or more attendants, and simple thrusts and sallies made toward it with a straw brush or with one's heavily-gloved hand. A sham

[1] John Simon Guggenheim Memorial Fellow, 1937–38.

[2] The physiological phases of these studies were carried out at the Barro Colorado Island Laboratory, C.Z., and the Gorgas Memorial Laboratory, Rep. Panama. The thanks of the authors are gratefully extended to Mr. James Zetek and Dr. Herbert C. Clark of these laboratories respectively for their unfailing courtesies during the investigations.

TABLE 1

Blood sugar and body temperature changes on emotional excitation of various tropical animals

ANIMAL USED	NUMBER OF CASES	BLOOD SUGAR			RECTAL TEMPERATURE	
		Normal range	Normal average	Average rise after emotion	Normal average	Average rise after emotion
		mgm. per cent	*mgm. per cent*	*per cent*	*°C.*	*°C.*
White-faced monkey *Cebus capucinus imitator*	7	87–132	105	23	39.8	0.3
Marmoset monkey *Leontocebus geoffroyi*	8	112–156	128	34	38.8	0.8
Black spider monkey *Ateles dariensis*	6	78–180	114	23	39.2	0.2
Red spider monkey *Ateles geoffroyi*	5	79–105	93	26	38.3	0.5
Night Monkey *Aotus zonalis*	3	100–184	130	35	38.5	0.8
Vampire bat *Desmodus rotundus murinus*	3	152–215	192	28	38.4	0.6
Fruit bat *Phyllostomus hastatus*	7	53–65	62	10	38.9	0.6
Short-tailed bat *Hemiderma perspicillatum*	6	60–65	63	9	37.5	0.7
Brown bat *Chilonycteris rubiginosa*	5	60–66	64	7	38.7	0.5
Ocelot *Felis pirrensis*	3	75–174	114	40	38.7	0.9
Raccoon *Procyon cancrivorus*	9	71–114	92	14	39.0	0.9
Kinkajou *Cercoleptes caudivolvulus*	3	67–185	117	21	37.2	1.5
Capybara *Hydrochoerus isthmicus*	6	146–279	219	16	37.9	0.9
Spiny rat *Proechimys semispinosus*	4	87–135	112	9	37.5	0.5
Mountain peccary *Tayassu pecari*	3	100–165	135	12	38.7	0.9
Collared peccary *Pecari angulatus*	8	98–196	134	18	38.5	1.3
Two-toed sloth *Choloepus hoffmanni*	11	57–120	79	54	34.6	0.5
Three-toed sloth *Bradypus griseus*	5	59–116	81	37	33.2	0.5
Armadillo *Dasypus novemcinctus*	4	54–97	72	24	31.9	1.1
Mexican opossum *Marmosa isthmica*	5	62–105	82	39	35.7	1.4
Zorro opossum *Didelphis marsupialis*	10	69–94	80	28	34.9	1.0
Water opossum *Chironcctes panamensis*	3	67–91	79	26	35.2	1.1
Crocodile *Crocodylus acutus*	24	76–138	101	25	27.4	1.4
Rattlesnake *Crotalus ruber*	2	67–90	79	16		
Fer-de-lance *Bothrops atrox*	4	63–87	73	11	31.2	1.1
Boa constrictor *Constrictor c. imperator*	4	54–80	70	14	27.3	1.4
Rainbow boa *Epicrates cenchris*	2	96–90	93	14	29.8	0.7
Coral snake *Micrurus nigrocinctus*	2	105–109	107	15	29.2	1.2

attack period of three minutes was used, except in a few instances in which somewhat more prolonged tests were carried out over six or nine minutes.

RESULTS. It is not within the province of this paper to describe in detail emotional reactions in different animals. Amongst the mammals, snarling and hissing, baring of the teeth, biting, clawing and aggressive charging were common characteristics. Monkeys manifested the greatest and sloths and armadillos the least emotional and general physical reactions. In the case of crocodiles, vigorous snapping of the jaws and lashing of the tail occurred, while snakes coiled and struck out frequently and made attempts to bite. In all cases, the greatest possible emotional response was drawn forth during the experimental period.

The results indicate that among mammals, the very active or vigorous forms show the highest normal blood sugar readings. Thus, the levels in monkeys, ocelots, kinkajous, peccaries and capybaras[3] generally ranged between 100 mgm. and 250 mgm. per cent. Relatively inactive forms, such as sloths, opossums and armadillos showed lower levels usually ranging between 60 mgm. and 100 mgm. per cent. Most of the snakes showed similar relatively low levels. Insectivorous and frugivorous bats showed the lowest normal blood sugar levels of all mammals (60 mgm. to 65 mgm. per cent). Carnivorous vampire (blood-eating) bats showed in contrast the highest blood sugar readings (approximately 200 mgm. per cent), with the exception of the capybara.

A consideration of the changes on excitation in various groups of animals shows that the very vigorous emotional responses of monkeys were correlated with large increments in blood glucose—from 20 to 35 per cent (average 28 per cent) in 29 cases. Blood sugar changes in opossums were nevertheless equally as great as in monkeys—average 29 per cent in 18 cases—although these animals were not nearly so active as the primates which were tested. The greatest hyperglycemic responses were observed in the case of sloths, especially those of the two-toed variety, notwithstanding the fact that these forms were very lethargic and gave little emotional display.

In most cases the affective and defensive reactions of sloths to the attack form of stimulation given above were confined, it may be observed, to gentle hissing and baring of the teeth, and occasional striking with the fore limb particularly on the part of the two-toed variety. Insectivorous and frugivorous bats were in contrast very aggressive, continually attempting to claw and bite and fly; and yet the latter animals showed the smallest alterations in blood sugar on excitation—on the average less than 9 per cent.

Analysis of the results shows that in the higher mammals (including monkeys, ocelots, vampire bats) the body temperatures ranged between

[3] This is a very vigorous although fat animal.

37° and 40°; in these there was an average blood sugar level of 104 mgm. per cent and an average rise on emotional activity of 22 per cent (range, 9–40 per cent, 68 cases). In the lower mammals (sloths, armadillos, opossums) the body temperatures ranged between 31° and 36°; and there was an average blood sugar level of 79 mgm. per cent and an increase on emotional activity of 37 per cent (range, 24–54 per cent, 38 cases).

In the emotional reactions shown above, apparently maximal changes in blood glucose and body temperature took place. It was found, at least, that doubling or tripling the excitation time (i.e., to 6 or 9 min.), in a number of cases, did not significantly affect the results.

The marked hyperglycemic reactions observed in the case of sloths are in contrast to the small amount of liver glycogen found in these animals—less than half the amount found in higher mammalian types (Britton, Kline and Silvette, 1938). However, these animals accumulate large amounts of food in the stomach (Wislocki, 1928), and even after a fasting period of three days or more the stomach usually contains considerable amounts of partly digested leaves and fruits having a high carbohydrate content (Britton). Further physiological data on some of the forms used in this study have already been published (Britton and Atkinson, 1938; Britton, Silvette and Kline, 1938).

It is recognized that blood glucose and body temperature levels may of course fluctuate rather quickly, and that the readings at any one time represent only the resultant balance from many metabolic exchanges. The results which were observed under uniformly controlled conditions in the present experiments are indicative, however, that certain responses characteristic of higher types are similarly present in many widely different as well as lower animal species.

SUMMARY

The higher mammalian tropical types, including monkeys, show a higher average blood glucose level and body temperature than lower types such as sloths and marsupials—on the average 104 mgm. per cent and 38.5° respectively, compared to 79 mgm. per cent and 34°.

Hyperglycemic reactions from emotional excitement were more marked in the lower mammalian forms, and body temperature rises also tended to be greater on excitation (except in sloths).

Sloths showed striking emotional hyperglycemic responses, but small changes in body temperature. In these animals the apparent psychic and general somatic expressions were of a low-grade character.

Blood-eating or vampire bats showed normal blood glucose levels three times as high as insectivorous and frugivorous bats—an average of 192 mgm. compared to 63 mgm. per cent. Further, emotional hyperglycemia was three times as great in the carnivorous bats.

Reptiles such as snakes and especially crocodiles showed emotional hyperglycemic and hyperthermal changes which were not greatly different from those observed in mammals.

There was no uniform correspondence apparent between general psychosomatic expressions and glycemic and thermal changes in individuals or in species.

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pH CHANGES IN ISCHEMIC HUMAN MUSCLE AFTER VOLUNTARY CONTRACTION[1]

GEORGE L. MAISON AND ARMAND C. FORSTER

From the Departments of Physiology and Surgery, St. Louis University School of Medicine, St. Louis, Mo.

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The following experiments attempted to reveal the relation of pH change to the genesis of the pain modality which occurs in intermittent claudication. Lewis, Pickering, and Rothschild (2) showed that a similar pain was produced in normal muscle by work without blood supply. They termed the modality ischemic pain and its cause factor P. Though many of the properties of factor P have been ascertained, its exact nature is not known. Due to the fact that lactic acid is the best known product of anaerobic contraction it has been widely suspected that either the lactate ion or pH change might be factor P. It has been shown that the pain is hastened by acidity and retarded by alkalinity (1).

It seems probable that the factor acts on nerve endings between the muscle fibers rather than within them—first, because the disappearance of the pain within a few seconds after return of blood supply would demand extremely rapid diffusion of the factor itself or of oxygen to reduce the concentration of the factor below the stimulating level in so short a time, and second, because so far as we know sensory nerve endings have not been described within the fibers.

If, then, factor P is a certain critical level of pH in the intercellular fluid it should be possible to reveal this relationship by charting the intercellular pH in a human muscle during the pain and its relief by return of blood supply.

A method for determining the intercellular pH in unanesthetized human subjects has been described (3). It consists briefly in the insertion of a capillary glass electrode into the belly of the muscle through a superficial incision. Local anesthesia is limited to the skin alone. This method permits continuous recording of the pH except during the actual contractions of the muscle.

We used the extensor digitorum communis weighted by means of an extensor ergograph which permitted variation of the load and measurement

[1] A preliminary report of these experiments appeared in the Proc. Soc. Exper. Biol. and Med. **38**: 425, 1938.

of the total work done. A sphygmomanometer cuff was placed on the subject's arm and inflated to a pressure of 160 to 200 mm. Hg when ischemia was desired.

The effect of ischemia alone on the pH of the intercellular fluid in human muscle. In seven subjects the greatest fall of pH as a result of lack of blood supply was a fall of 0.05 pH unit in seven minutes. Table 1 shows the summarized data.

The effect of ischemic work on the pH of intercellular fluid. During the actual work no recording can be made, but immediately on cessation of contraction the instrument was returned to operation, and simultaneously in these experiments the occlusive pressure was released. At this moment the pH was found to be falling rapidly. Usually, within one minute the pH reached a minimum value at which a temporary plateau occurred followed by recovery.

TABLE 1

Effect of ischemia alone on intercellular pH of human muscle at rest

Minimum brachial pressure 160 mm. Hg

SUBJECT	TIME	pH FALL	SUBJECT	TIME	pH FALL
	min.			*min.*	
J. B.	5½	0.01	J. D.	7½	0.05
W. A.	7	0.05	S. A.	10	0.06
O. B.	6½	0.00	E. C.	11½	0.04
L. B.	6½	0.03			

Average pH fall per minute (calculated from above data)—0.004 pH.

The extent of pH fall from the resting baseline to the minimum value is tabulated in table 2. There is a very general and crude correlation between the degree of pH fall and the amount of work done in different subjects. It is to be noted that work was carried to or beyond the pain producing level in each trial.

A typical graph of one experiment is shown in figure 1. The time scale in this graph is too gross to permit showing any readings before the minimum. However, the details of the immediate post work changes are typified by the first portion of figure 2.

The time relations of pain and intercellular acidity. In each trial the subject worked the ischemic arm until pain was very definite. Simultaneously the pressure around the arm was released and pH recording resumed. The subject then announced when pain was gone and time was measured from this moment until pH reached minimum. In seven subjects the average duration of increasing acidity after pain was relieved was 23 seconds. In table 3 the trials are summarized. These figures do not reveal the total time during which the pH was lower than that which

existed when the pain was felt since they include neither the plateau nor the rising phase of the curve.

Demonstration of dissociation of pain and intercellular pH by a different type of experiment. Figure 2 shows a detailed graph of a variation of the experimental procedure. Here ischemic work was done until pain resulted, then ischemia was relieved and pH followed until the typical minimum plateau was reached. Pressure was then reapplied, and the pH

TABLE 2

Degree of fall of intercellular pH in human extensor digitorum communis as a result of ischemic work

The blood supply was interrupted when work started and was restored immediately at the cessation of work. Twenty to 50 minutes' rest was permitted between work trials.

WORK DONE	CONTRACTIONS PER MINUTE	LOAD	APPROXIMATE DURATION OF WORK	pH FALL	TIME REQUIRED FOR pH RECOVERY	SUBJECT
kgm. cm.		*gm.*	*sec.*		*min.*	
46	90	300	40	0.08	14	L. B.
51	90	300	35	0.07	16	L. B.
94	90	600	35	0.10	10	L. B.
123	60	600	50	0.25	19	S. A.
154	60	600	65	0.28	15	S. A.
157	60	600	65	0.17	19	S. A.
166	60	600	50	0.17	26	E. C.
175	60	600	50	0.16	15	O. B.
186	60	600	55	0.18	31	E. C.
192	60	600	55	0.11	16	O. B.
200	60	600		0.16	17	W. A.
200	60	600	60	0.14	16	O. B.
213	60	600	70	0.18		E. C.
250	60	600	45	0.37	20	W. A.
255	60	600	70	0.29	25	O. B.
300	90	600	50	0.22	23	J. D.
300	90	600	50	0.22	29	J. D.
300	60	600	60	0.32	20	W. A.
395	90	600	80	0.26	16	J. B.
425	90	600	90	0.35	16	J. B.

again fell markedly far below the previous plateau, yet pain did not reappear. After a time the pressure was released and 6 seconds later the instrument showed the start of the rise in pH due to return of blood supply. This is a good example of the small duration of the inherent latency of the glass electrode system.

Control experiment. Validification of the present method has been published previously (3). During this series of experiments accidental misplacement of the capillary glass electrode resulted in another control:

right beside the extensor digitorum communis lies the extensor carpi radialis longus. By accident the capillary was inserted into the belly of the latter muscle. Work was done in the usual way by the extensor of the

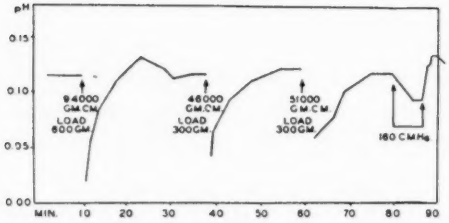

Fig. 1

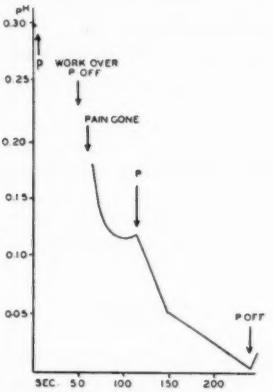

Fig. 2

Fig. 1. Plot of pH changes in human extensor digitorum communis. Subject, L. B. The ordinate is in pH units from an arbitrary zero. Work is done with the arm rendered ischemic. Rate of contractions 90 per minute. Each work bout produced pain. Blood supply is occluded just before work starts and restored at the instant work is stopped. No pH readings are plotted in this graph from start of work until pH reaches minimum about 1 minute after work ceases. The last fall in this graph was due to ischemia alone without work.

Fig. 2. Detailed graph of pH changes from the instant work ceased. P = pressure (160 mm. Hg). Single dot near top of ordinate indicates pre-work pH level. Note that pain is gone before pH reaches minimum. In this case after the minimum pH with blood supply was reached blood supply was again interrupted and a marked further fall of pH occurred but no pain resulted. Latent period from final release of pressure to start of upswing of pH was 6 seconds. Subject E. C.

TABLE 3

Time in seconds from disappearance of ischemic pain until minimum intercellular acidity was reached

SUBJECT	TRIALS	AVERAGE	SUBJECT	TRIALS	AVERAGE
J. B.	1	30	J. D.	2	24
W. A.	3	18	S. A.	3	24
O. B.	3	15	E. C.	3	29
L. B.	2	18			

digits and the resulting change in pH in the extensor of the wrist is shown in figure 3A. The electrode was then properly placed in the extensor of the digit and the usual results followed as seen in figure 3B.

Pattern of reaction changes after voluntary activity of human muscle. From the results of previous experiments on man (3) a pattern has been formulated: Mild work with blood supply results in an alkaline change followed by recovery. Moderate work with blood supply produces an alkaline shift rapidly obliterated by an acid change. Prolonged heavy work at its end leaves the muscle more acid than normal and acidification may continue for as much as 120 seconds after work ceases. Recovery to normal pH is a long slow process which may require 20 minutes or more. From the present results we can add to this that a state of ischemia during the work heightens the acidification resulting. If the blood supply is restored at the end of work the time required for the intercellular fluid to cease

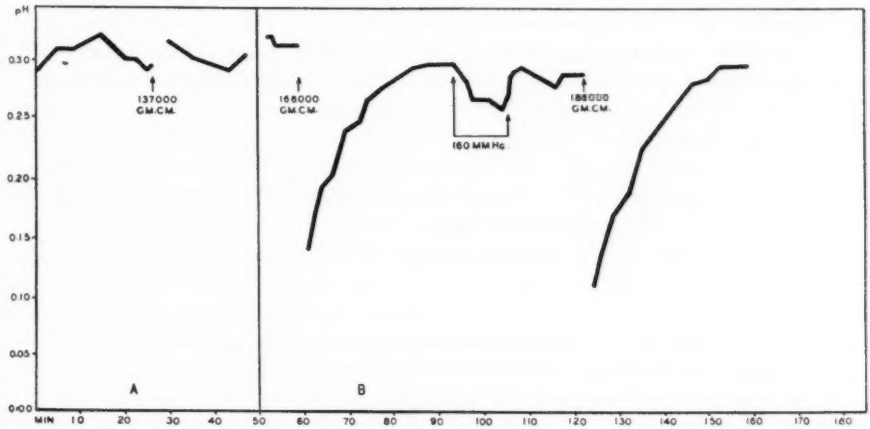

Fig. 3A. Electrode was in extensor carpi radialis longus. Work was done by extensor digitorum communis. Conditions as in figure 1.

3B. Electrode placed in extensor digitorum communis with typical results. Subject E. C.

becoming more acid is much the same as if the blood supply had been present throughout. Only the degree of acidification is changed. Contrarily, if the ischemia continues after the work is done both the degree and duration of the acidification are markedly increased.

Interpretation of relationship between pH and ischemic pain. Since the intercellular pH continues to fall after pain has disappeared factor P can hardly be a certain critical level of pH in the intercellular fluid. This is strongly emphasized also by the marked acidification during secondary ischemia after pain has been relieved.

This evidence does not rule out the possibility that factor P may be a certain rate of pH change in the intercellular fluid. However, on this basis it is hard to explain the fact that in ischemic work once pain is produced

it is unrelieved until the blood supply is restored. It is hardly conceivable that the pH can continue to fall over so long a period at a rate as rapid as this would imply.

A second possibility is that factor P does not act in the intercellular spaces but intracellularly. If this were so pH change might be factor P since there is no proof at present that inter- and intra-cellular pH's are identical. However, nerve fibers have not been demonstrated in the muscle fibers as yet.

CONCLUSIONS

1. The intercellular fluid of human voluntary muscle during 5 to 10 minutes of ischemia at rest showed no change greater than 0.06 pH in 7 subjects.

2. When ischemic muscle is voluntarily worked until pain appears, restoration of blood supply relieves the pain within 10 seconds yet maximal acidity of the intercellular fluid does not occur until 23 seconds later (average time from 17 trials).

3. A routine is described whereby a degree of acidity of the intercellular fluid far greater than that present when ischemic pain exists can be produced without the occurrence of pain.

4. These data suggest that the acidity of the intercellular fluid cannot be factor P.

The authors wish to express their appreciation to the medical students of St. Louis University for their kindness in volunteering as subjects.

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HYPERPARATHYROIDISM PRODUCED BY DIET

EMIL J. BAUMANN AND DAVID B. SPRINSON

From the Laboratory Division, Montefiore Hospital, New York

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In the course of some recent experiments, we had occasion to change the diet of our rabbits from the stock ration of alfalfa, oats and occasional greens to carrots and oats. When after several months these rabbits came to autopsy we found greatly enlarged parathyroid glands. To determine whether this hypertrophy was due to the change in diet or to some other environmental factor, additional rabbits were placed on this diet of carrots and oats, and after 3 months or more their external parathyroids were found to weigh 30 to 50 mgm., and in one instance 189 mgm., whereas on our stock diet, they seldom weigh more than 10 to 15 mgm. Histological examination showed that the cells and nuclei were often 50 per cent larger than normal and that the cytoplasm contained much more fat. In those rabbits on this diet for more than 6 months, the lipids accumulate in the cells in single large droplets which distend the cell bodies and distort and dislocate the nuclei. These anatomical changes indicated increased activity, at least at some time during the development of this parathyroid hypertrophy. It has been observed in every one of the 25 rabbits studied.

Occasionally, on the carrot and oats diet, the inorganic phosphate[1] in the serum of adult rabbits tended to be lower than normal, sometimes less than 2.5 mgm. per 100 cc., but for the most part it was between 3 and 4.5 mgm. per cent. Serum Ca, which is 13 to 15 mgm. per cent on the alfalfa and oats diet, decreased on carrots and oats, sometimes to less than 11 mgm. per cent. But on the whole, serum Ca and P were both within the rather wide range of normality shown by rabbits (see table 1).

Serum phosphatase which is influenced, although probably not directly, by variations in the degree of parathyroid function, was low. In adults it is difficult to establish a reduction because the amount of serum phosphatase is small in mature animals. In one series of rabbits (3 male and 4 female) about 10 months old, the maximum, minimum and average serum phosphatase values on the alfalfa and oats diet were 2.1, 0.6 and 1.4 units (milligram of inorganic P liberated per 100 cc. per hour) respectively, while after 1 month on the carrot and oats diet, the corresponding figures were

[1] Serum calcium was determined by the method of Clark and Collip (1), inorganic phosphate and phosphatase according to Bodansky (2).

1.1, 0 and 0.6. In another series of 7 older adult rabbits, after 5 months' feeding with carrots and oats, the maximum, minimum and average serum phosphatase values were 0.5, 0 and 0.2 units respectively. During the time that the serum phosphatase was diminished the parathyroids were very active as indicated by their hypertrophy and the great increase in amount of circulating parathormone, as we shall presently show. The serum Ca, P and phosphatase measurements were made on 24 rabbits. A few of the data are shown in table 1.

TABLE 1

Serum Ca, P and phosphatase of rabbits on the carrot and oats diet

RABBIT NO.	TIME ON CARROT AND OATS DIET	Ca	P	PHOSPHATASE
	months	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*units per 100 cc.*
1	0*	14.8	3.2	1.1
	1	13.8	2.8	1.1
	7	10.8	2.7	
2	0*	14.2	3.6	1.6
	1	14.0	3.5	0.7
	5	12.3	2.8	
	7	11.6	3.9	
3	0*	13.7	3.0	1.7
	0.5		2.8	0.7
	1		3.1	0.0
	9	10.5	3.7	
4	0*	13.4	3.4	2.1
	0.5		3.7	0.8
	1	13.4	3.3	0.5
	5	12.5	3.1	
5	0*	15.4	3.0	1.1
	0.5	14.3	3.3	0.6
	1	12.8	3.3	0.5

* At the time of the first series of observations on each rabbit, the diet was alfalfa and oats.

We were able to shed more light on this problem by using the Hamilton and Schwartz test (3) (4) for parathormone, which, briefly, is carried out as follows: 100 mgm. of Ca as $CaCl_2$ in 10 cc. of water is given to an adult rabbit by stomach tube at the beginning of the test and again 1, 3 and 5 hours thereafter. The substance to be tested is injected intramuscularly at the beginning of the test. Blood is taken for serum Ca determinations at the beginning of the test and 7 to 15 minutes after the last 2 administrations of $CaCl_2$ solution. The curve of serum Ca so obtained is compared

with those previously determined in control animals and in those injected with known amounts of parathyroid hormone. In normal adult rabbits, the serum Ca rises to 15 or 16 mgm. per cent after the second dose of $CaCl_2$ is administered—that is the "1st hour" specimen—and returns to, or nearly to, the normal value and remains so after the 3rd and 5th hour administrations of $CaCl_2$. That is, successive feedings of $CaCl_2$ produce smaller rises in the serum Ca of rabbits. Hamilton and Schwartz regard as normal any rise of serum Ca at either the 3rd or 5th hour below 1.2 mgm. per cent. When 5 to 7 Hansen units of parathormone per kilogram were given they found a maximum rise of serum Ca of about 2 or 3 mgm. per cent at the 3rd or 5th hour; with a dose of 10 or 20 units per kgm. the maximum rise was 2.5 to 4.0 mgm. per cent. We have confirmed the observations of Hamilton and Schwartz as to the validity of the test as a measure of parathyroid hormone. *a.* In 9 normal adult rabbits the maximum rise in serum Ca at either the 3rd or 5th hour varied from 0 to 1.0 mgm. per cent, the average being 0.5. *b.* Administration of varying doses of parathyroid extract gave responses similar to those of Hamilton and Schwartz, although only roughly proportional to the dose. *c.* In the 3rd week of pregnancy in a rabbit the maximum rise was 1.2 mgm. per cent. Hamilton, Dasef, Highman and Schwartz (5) found that during the latter part of pregnancy in man there is an increase in circulating hormone of the parathyroid measured in this way. As a consequence of these and other trials, as well as from the experience of Hamilton and others with the clinical application of this test, we felt justified in using it in our animals as a measure of circulating parathyroid secretion.

When this test was performed on rabbits on the carrot and oats diet, all showed evidence of an increased amount of circulating parathyroid secretion, corresponding to the effect produced by the injection of 15 to 25 units of parathormone per kilogram as a rule, though not infrequently a response equivalent to an injection of as much as 40 or more Hansen units per kilogram was seen, especially in immature animals. The reaction to this diet begins within one week and has continued at about the same degree of intensity for $2\frac{1}{2}$ years in 3 rabbits and for a year in another group of 6. In all, Hamilton tests have been made frequently on 23 rabbits on the carrot and oats diet and every test indicated an abnormally large amount of circulating parathyroid hormone. Such responses were never obtained from rabbits fed alfalfa and oats. Chart 1 shows the serum calcium curves obtained on 2 rabbits on the alfalfa and oats diet and also on the same animals after one week on the carrot and oats diet.

Diarrhea often develops when too large a proportion of carrots is fed. This can be controlled by reducing the carrots and increasing the oats in the ration. During periods of diarrhea the coats of the animals become dull; resistance to infections appears to be lowered. A slight anemia

develops, if one may judge from the proportion of serum obtained by centrifuging the blood. On the hay and oats diet slightly less than 50 per cent of serum is obtained, whereas on the carrot and oats diet, 55 to 60 per cent of serum can be separated from the blood.

The cause of the parathyroid hypertrophy and the resulting chronic hyperparathyroidism was found to be in the Ca:P ratio of the diet. Whereas our stock diet of alfalfa, oats and occasional greens, on which rabbits will live and reproduce normally, has a Ca:P ratio of about 4, it is 0.5 for the carrot and oats ration. On adding enough $CaCO_3$ to the latter diet to

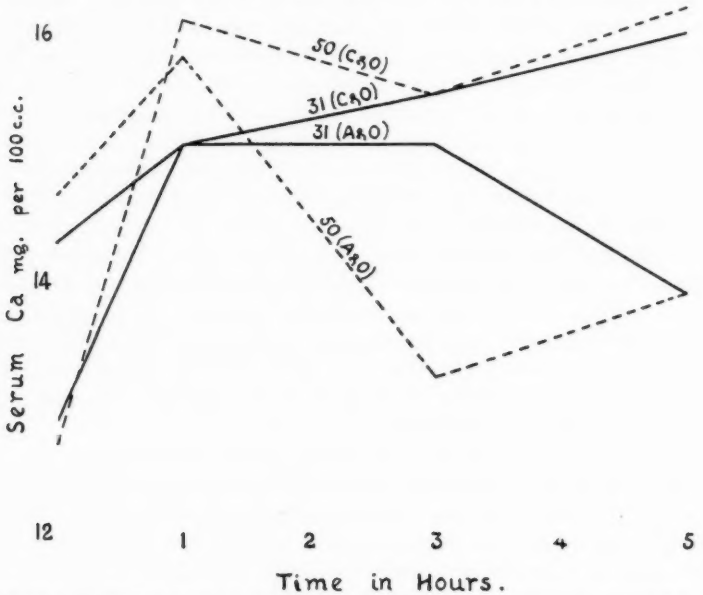

Chart 1. Hamilton tests on rabbits on a diet of alfalfa and oats compared to that of carrots and oats.

make this ratio one, the rabbits responded to the Hamilton test as they do on alfalfa and oats. Table 2 gives some of the data of this experiment.

Parathyroid hypertrophy has frequently been observed in cases of rickets in man and other animals. Hamilton and Schwartz (6) have shown that young rabbits made rachitic with a high Ca low P diet have more circulating parathormone than do normal rabbits. Our stock diet of alfalfa, oats and occasional greens has similar proportions of Ca and P, but it evidently contains sufficient vitamin D to prevent the development of rickets; the parathyroids are not enlarged and the Hamilton and Schwartz test for parathormone is negative. On the other hand, on the

low Ca high P carrot and oats diet, we have shown that rabbits have a greatly increased amount of circulating parathormone. Drake, Albright and Castleman (7) have produced enlarged parathyroids in rabbits in a somewhat similar way by parenteral administration thrice daily of 25 mgm. of P as a neutral sodium phosphate solution, giving in effect a diet with a low Ca:P ratio. They described the glands as hyperplastic but not hypertrophied because the size of the cells showed no increase over their normals.

Histological examination of the long bones of these rabbits did not show any outstanding differences from the bones of those animals fed with alfalfa and oats, except that the blood vessel spaces were considerably larger—an indication of greater vascularity. Since changes in the structure of bones by excessive dosage with parathyroid extract have been produced only in young animals, and since the animals used in these

TABLE 2

The effect of calcium feeding on the amount of circulating parathyroid hormone in rabbits on a carrot and oats diet, as shown by the Hamilton test

TIME ON CARROT AND OATS DIET	MAXIMUM CALCIUM RISE				
	6	2	3	1	4
months	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
3	3.6	2.8	2.0	2.3	0.9
5	2.8	4.0	3.2	3.2	1.6
	Between 5 and 6 months, fed 2.5 grams $CaCO_3$ daily			No $CaCO_3$ fed—controls	
6*	1.0	0.2	0.4	4.0	1.4
7	3.7	3.6	3.0	4.0	1.9
9	4.2	4.3	3.8	3.8	

* After these observations were made, $CaCO_3$ feeding was stopped.

experiments were all adults such alterations would not be expected. Chemical analysis of the tibias for ash, Ca, P and Mg did not disclose any significant differences between animals on these diets.

We have then, in this low Ca high P diet of carrots and oats, a means of producing simply and regularly a severe chronic hyperparathyroidism. If one may judge from the Hamilton and Schwartz test, the reaction is equivalent to that caused by injection of 15 to 30 or more Hansen units per kilogram of parathormone in adult rabbits, while in those 3 to 6 months old the response is twice as great as in the mature animals. Moreover, the effect of a single injection of parathyroid extract wears off after several hours and after 10 to 14 days repeated injections become less and less effective, whereas this ration keeps rabbits continuously in a state of increased parathyroid activity. We have at this time 3 rabbits that now, after having been kept on this diet for $2\frac{1}{2}$ years, still respond to the Hamil-

ton test as would rabbits that had been injected with 20 to 30 Hansen units of parathormone per kilogram.

SUMMARY

Hyperparathyroidism in rabbits has been produced by feeding a low Ca high P diet. This diet causes enlargement of the parathyroids to two or more times their normal size and hypertrophy of the cells and nuclei with an increase in the amount of lipids in the cytoplasm. Serum Ca and P are low but within the normal range, while serum phosphatase is possibly less than normal. Aside from increased vascularity no important changes were found regularly in the bones. The Hamilton and Schwartz test for parathormone is always high.

The postmortem and histological examinations were made by Dr. David Marine, to whom we are very grateful.

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BODY SIZE AND ENERGY METABOLISM IN GROWTH HORMONE[1] RATS[2]

MAX KLEIBER AND H. H. COLE

College of Agriculture, University of California, Davis

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The question which we tried to answer by the investigation reported here may be formulated as follows: Is it better to regard the metabolic rate of an animal as the result of a somatogenic adaptation to a condition of its body as a whole, such as its body size, or as a genetically fixed characteristic of its tissues?

This question seemed solved when in 1925 Terroine and Roche in France and Grafe, Reinwein and Singer in Germany published the rule that the metabolic rate per unit weight of homologous tissues in vitro is essentially the same for tissues from large and small animals, whereas in vivo the metabolic rate per unit weight of large animals is systematically smaller than that of small animals. Thus the metabolic rate of the animal cannot depend on the rate of tissue metabolism as measured in vitro, but must be the result of a regulatory influence of the body as a whole. This rule of the uniformity of tissue metabolism has, however, been challenged by various authors; some of the literature dealing with this question has recently been discussed by O. Busse (1938).

The alternative to Terroine's rule, which Wels (1925) accredits to Rubner, namely, the idea that the metabolic rate per unit weight is a fundamental characteristic of a species ("fundamentale Arteigenschaft") can hardly be accepted since this rate is the same for women and goats and differs systematically between large and small dogs (Rubner, 1883) and between large and small rabbits (Richet, 1889). One might, however, still argue that the differences in these rates between large and small individuals of the same species could be genetically determined since large and small dogs, as well as large and small rabbits, belonged to different races. It may also be argued that the exceptionally large rats studied by

[1] "Growth hormone" is used here as a convenient name for the growth promoting pituitary extract prepared according to Evans et al. (1933) and is not meant to express any view with regard to the chemical nature, the physiological purity or the mode of action of this extract.

[2] A preliminary report on the subject of this paper was presented at the 16th International Congress of Physiologists in Zurich, Switzerland, August 19, 1938.

Benedict, Horst and Mendel (1932) might have been genetically different from the control rats of normal size.

In order to distinguish between genetical predetermination and somatogenic adaptation, we compared the metabolic rates of rats made giant by injections of Evans' growth hormone with those of normal-sized littermate controls. Metabolism trials on growth hormone rats have already been carried out by Lee, Teel and Gagnon (1929). These authors compared the metabolic rate of 4 injected rats with that of 4 controls chosen as large as possible "in order to obviate as much as possible the effect of size per se on metabolism." Our aim was, to the contrary, to study the effect of "size per se" if such an effect should exist. Our data on fasting catabolism were supplemented by the measurements of the oxygen consumption of the surviving diaphragm in vitro after the rats had been killed. The authors gladly acknowledge the technical assistance of A. H. Smith.

METHOD. *a. Rats.* The rats used for these experiments were reared in our colony from the Long-Evans strain. Four of the rats used were born March 3, 1937, the others from April 13 to 21, 1937. The rats were kept in groups of 2 to 3 together in wire cages in a colony the temperature of which was regulated between 18 and 24°C.

Daily intraperitoneal injections of 1 cc. of growth hormone prepared according to the method of Evans et al. (1933) were started May 5 for the two oldest rats, June 11 for 7, June 15 for 3, and June 22 for 10 rats. Littermates were kept as controls under the same conditions as the injected rats. There were 20 controls for 22 injected rats.

Three injected rats and their controls were killed between November 18 and 24 in order to obtain a chemical and anatomical analysis of the rats at this stage of the experiments. At the same time, the hormone injections were discontinued in 12 of the injected rats in order to study their metabolic rate independent from the direct effect of the hormone. In 7 rats the daily injections were continued until January 4, 1938. The time when the rats were killed will be mentioned in the description of the respiration trials.

b. Feed. Except during the night prior to the respiration trials the rats were fed ad libitum: The food contained whole wheat 67.5, casein 15.0, whole milk powder 10.0, butter 5.2, NaCl 0.8 and $CaCO_3$ 1.5 per cent.

c. Respiration trials. The fasting catabolism of all rats was measured in a first series of respiration trials from November 4 to 10, 1937. These measurements were repeated in a second series November 18 to 24. The metabolic rate of the 36 remaining rats (three pairs had been sacrificed after the second series of respiration trials) was measured again in 3 further series of respiration trials December 9–15, January 9–15, and January 16–22. During the last mentioned (5th) series of trials 7 pairs of rats were killed; the O_2 consumption of the diaphragms in vitro was measured; and the carcasses were dried for chemical analysis after some anatomical

measurements had been secured (see below). With the remaining 12 pairs of rats a new (6th) series of respiration trials was carried out from January 24–28, during which period four pairs of rats were sacrificed. A 7th, 8th and 9th series of respiration trials were then carried out with 8, 5 and 2 pairs of rats respectively. The last pair of rats was sacrificed after the respiration trial February 28.

The CO_2 production and O_2 consumption of the rats were measured in an apparatus which had been designed and constructed at our station several years ago for serial work with small animals (Kleiber, 1939). It permits the simultaneous measurement of the respiratory exchange of 7 separate rats during a 6-hour period.

The 7 rats whose fasting catabolism was to be measured were taken from the rat colony at 5 p.m. and brought to an air-conditioned chamber, adjusted to a temperature of approximately 30°C. The rats from then on had no food but had access to water. The experimental procedure during the next day may be illustrated by a record out of the work book. The time is given in hours of a 24-hour day starting at midnight.

Time (January 21)

8:00 Rats weighed in colony and then brought back to air-conditioned room (30°).

9:30–10:00 Rats moved from air-conditioned chamber to their respective respiration chambers (left open) in respiratory cabinet held at 30°C. Water of 30°C filled into seals and on top (glass windows) of respiration chambers. O_2 filled into gas burettes of respiration apparatus. Cabinet ventilated with air stream from compressor.

10:30 Respiration chambers closed; absorption of CO_2 started.

11:00 Reading of water menisci in gas burettes (O_2 consumption) started.

15:00 End of respiration trials. Rats removed from respiration chambers, weighed, and vaginal smear made. Rats brought back to their cages in rat colony.

15:23 Injected rat B2717 killed (knocking head on table).

15:26 Pieces of diaphragm of this rat in buffered glucose—Ringer[3] solution kept at 37°C. with O_2 bubbling through solution.

15:30 Diaphragm pieces are in Ringer solution in Warburg vessels, vessels connected to manometers, O_2 streaming through vessels for ½ minute. Manometers installed on shaker of thermostat and shaking is started.

16:15 First reading of O_2 consumption in vitro. Readings every 15 minutes.

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[3] The solution was mixed as follows:

100 cc. of a balanced ion solution [Dixon (1934) p. 63] which contained per liter:

KCl	0.008 gram
$CaCl_2$	0.005 gram
NaCl	8.5 gram
glucose	1.9 gram

and 10 cc. of a buffer solution which contained per liter:

Na_2HPO_4	0.12 mol
$KHPO_4$	0.02 mol

and had a pH of 7.4 (checked with the electrode).

17:45 Last reading of O_2 consumption, diaphragms removed from Warburg vessels, superficially dried, laid on paper, their outline drawn for determination of surface area, then put into drying dishes, weighed, and put into drying oven at 105°C. for determination of dry matter.

The CO_2 production of the rats was determined after the respiration trials by titration of the alkaline solution in the absorbing batteries of the respiration apparatus.

During the measurements of the tissue respiration in vitro, the killed rats were skinned, the skin laid on paper, and its outline drawn. The figure was then cut out and weighed for the determination of the skin area. Several linear measurements were taken (nose-anus, nose-tail, width and length of head and thickness of femur). The skin, heart, liver, thyroid gland, and adrenal glands (the latter only in 8 pairs) as well as the remainder of the carcass, were weighed and then dried at 110–115°C.[4] The liver and the remainder of the carcass were analyzed for fat, protein, and ash.

The number of respiration trials carried out in the 9 series is 127 on injected and 118 on control rats.

RESULTS. *a. Influence of injections on growth.* When the injections were started, the rats had an average body weight of approximately 200 grams. Five months later when the injections were stopped in one group the average weight of two groups of injected rats was 460 and 500 grams respectively; the corresponding groups of controls weighed 280 and 310 grams respectively. The difference between the average weight of the two groups of controls and a similar difference between the average weight of the two corresponding groups of injected rats appeared consistently during the entire period of injection. One out of the 22 injected rats did not respond to the injection of the hormone, a phenomenon observed previously by Evans et al. The data obtained on this rat are excluded from the averages reported in this paper. After the cessation of the injections the body weight of the injected rats dropped considerably, reaching a plateau of 400 grams approximately 4 weeks after the last injection. The average weight of the rats just before killing was 397 grams for the injected rats and 282 for the controls (table 1). The former mean includes one rat killed 2 weeks after the last injection, one rat killed 4 weeks, and 16 rats killed between 5 and 10 weeks after the last injection.

The cubic root of the body weight as a general basis for comparing linear dimensions was on the average 12 per cent greater in the injected rats than in the controls. The body length (nose-anus) of the injected rats was only 10 per cent, the tail length only 7 per cent, and the length of

[4] The first three rats were dried at 105°C. but it was found that in this case constant weight could not be reached in 20 hours, which was necessary for a continuous procedure.

the head only 5 per cent in excess of the respective dimensions in the controls. These results indicate that the injections stimulated true but heterogonic growth. The injected rats were stockier than the controls. The average relative skin area, however, was the same for injected rats and controls, namely, 7.58 and 7.54 dm.^{2} per unit of the $\frac{2}{3}$ power of body weight in kilograms respectively.

Table 1 shows the moist weights of various organs in injected rats and controls. The last column indicates that the liver was slightly and the heart considerable hypogonic, the latter being only 15 instead of 41 per cent heavier in the injected rats than in the controls. The skin of the injected rats shows slight hypergony. The moist thyroid gland of the injected rats was on the average only 15 per cent (instead of 41) heavier than that of the controls. It is suggestive to relate this hypogony of the thyroid gland to the antithyrotropic effect (resulting from injections of rat

TABLE 1

Weight of moist body and organs

	WEIGHT		INCREASE ABOVE CONTROL	
	18 injected rats	16 control rats	Absolute	Per cent of controls
	grams	*grams*	*grams*	*per cent*
Body weight just before killing	397	282	115	41
Weight of liver	12.0	8.8	3.2	36
Weight of heart	1.00	.87	.13	15
Weight of skin	74.7	49.0	25.7	53
	mgm.	*mgm.*	*mgm.*	
Endocrine glands				
Weight of thyroid gland	24.7	21.4	3.3	15
Weight of adrenal glands (8 pairs only)	45.2	61.4	-16.2 ± 5.8	-27

pituitary extracts) which has recently been reported by Anderson and Evans (1938).

The moist adrenal glands of the injected rats were on the average 16.2 mgm. lighter than those of the controls. If the weight of an organ can be taken as a characteristic for tropic action of hormones, then our result would indicate an antiadrenotropic effect resulting from the chronic administration of the pituitary extract used in our trials.

In order to investigate the statistical significance of the heterogony in the development of our injected rats as compared with the controls the moist and dry weights of the various organs have been expressed in per cent of the moist and dry weights of the body of each rat. The averages of these relative weights together with their standard deviations, are given in table 2. The differences in the relative liver and skin weights are not statistically significant. The hypogony of the heart is, however, signifi-

cant especially when dry weights are compared. The difference between the relative weights of the moist thyroid glands in the injected rats and controls, namely, 1.4 ± 0.5 milligram per 100 grams body weight, has a random probability of only 1 per cent and is thus decidedly significant.

TABLE 2

Dry matter and relative weight of organs

	DRY MATTER CONTENT		RELATIVE WEIGHT			
			Weight of moist organ in terms of weight of moist body		Weight of dry organ in terms of weight of dry body	
	Injected rats	Control rats	Injected rats	Control rats	Injected rats	Control rats
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Body just before killing	41.6	37.6				
Liver	31.7	30.7	3.07 ± 0.13	3.16 ± 0.15	2.39 ± 0.16	2.51 ± 0.12
Heart	22.8	22.6	0.256 ± 0.006	0.318 ± 0.015	0.141 ± 0.004	0.192 ± 0.006
Skin			19.1 ± 0.7	17.8 ± 0.7	26.9 ± 1.2	24.6 ± 0.8
			mgm. per 100 grams	*mgm. per 100 grams*	*mgm. per 100 grams*	*mgm. per 100 grams*
Thyroid gland	34.4	30.5	6.4 ± 0.3	7.8 ± 0.4	5.2 ± 0.2	6.2 ± 0.3
Adrenal glands	39.9	33.7	11.4 ± 1.3	21.9 ± 1.5	11.5 ± 1.3	19.8 ± 1.5

TABLE 3

Chemical composition of rats after cessation of injections

	18 INJECTED RATS	16 CONTROL RATS	DIFFERENCE IS STATISTICALLY SIGNIFICANT
Weight of empty* carcass (grams)	390 ± 12	277 ± 8	Yes
Water in empty* carcass (per cent)	57.9 ± 0.7	61.4 ± 0.5	Yes
Composition of dry carcass:			
Ash (per cent)	11.6 ± 0.3	12.4 ± 0.3	No
Fat (per cent)	36.6 ± 1.2	34.0 ± 1.4	No
Protein (+ glycogen) (per cent)	51.8 ± 1.0	53.6 ± 1.2	No
Protein factor† $\left(\frac{\text{Protein by difference}}{\text{N(Kjeldahl)}}\right)$	6.12 ± 0.015	6.14 ± 0.018	No

* Empty = without intestinal content.

† The factor for Myosin according to the Chittenden and Cummins analysis would be 5.96.

The same is true for the difference in the relative dry weights. The difference in the relative weights (moist and dry) of the adrenal glands of injected and control rats is statistically highly significant.

b. Chemical composition of growth hormone rats and controls. Table 3

shows the chemical composition of injected rats sacrificed after cessation of injection and their controls. The water content of the control rats (61.4 per cent) is the same as that reported for control rats by Bierring and Nielsen (1932) (61.5 per cent). The relative water content of our injected rats however, was lower than that of the controls, and the difference (3.5 ± 0.86 per cent) is statistically highly significant, whereas Bierring and Nielsen found that their growth hormone injected rats had a higher water content than the controls. It can be inferred from their data that they killed their rats during the period of injection. The discrepancy between their results and ours may then be explained by the period without injections which preceded the killing of our rats lasting 2 weeks for one, 4 weeks for one, and 5 or more weeks for 16 rats. During this post injection period the injected rats lost more than 10 per cent of their body weight.

If it is assumed that at the end of the period of injections our injected rats had the same water percentage in their bodies as those of Bierring and Nielsen, namely, 63.5 per cent, then with an average body weight of 461 grams their average water content at the end of injections would have been $461 \times 0.635 = 293$ grams. At the time of killing, the rats had an average weight of 398 grams and contained 58 per cent of water; their average water content was thus $0.58 \times 398 = 231$ grams. The average loss of water after the cessation of injections would then have amounted to $293 - 231 = 62$ grams. The loss of body weight was $461 - 398 = 63$ grams; the loss in body weight after cessation of injections would thus have been entirely a loss of water. Bierring and Nielsen calculate that the loss after cessation of injection was only 60 per cent water; this calculation was, however, based on the assumption that the injected rats after the loss had the same water percentage as the controls, which assumption is not confirmed by our observations.

The difference in the ash, fat and protein content in the dry matter of injected and control rats is not statistically significant.

c. Effect of injections on fasting catabolism. The respiratory quotient of the injected rats was 0.735 ± 0.0017 (mean of 124 determinations), that of the corresponding controls 0.732 ± 0.0006 (mean of 115 determinations). The difference has a random probability of a little less than 3 per cent and can therefore be regarded as statistically significant. The slightly higher R. Q. of the injected rats may suggest the idea that 18 hours of fasting are relatively speaking not quite as thorough a fast for the injected giant rats as they are for the controls.

The results of our respiration trials are summarized in table 4. Forty-two results on injected rats and 40 on controls were obtained in two series of respiration trials carried out during the period when the rats were injected daily with pituitary extract. During this period the injected rats weighed on the average 64 per cent more than the controls. The fasting

catabolism[5] per animal of the injected rats, however, was only 26 per cent higher than that of the controls. The fasting catabolism of the control rats was 73.7 kilocalories per unit of the $\frac{3}{4}$ power of body weight in kilograms. This approximates very closely the mean of 71.8 kilocalories per kgm.$^{3/4}$ resulting from a survey that included a 150 gram ring dove and a 679 kilogram steer (Kleiber, 1932).

The fasting catabolism per unit weight of the injected rats is only 77 per cent of the corresponding figure for the controls. Even per unit body surface (expressed as units of the $\frac{2}{3}$ power of body weight) the metabolic

TABLE 4

Metabolic rate per various units of body size

	INJECTED RATS	CONTROL RATS	INJECTED IN PER CENT OF CONTROL
Period I: During injections			
Number of determinations	42	40	
Average body weight during respiration trials (grams)	452	275	164
Average fasting catabolism per day per rat (kilocals)	35.3	28.0	126
Average fasting catabolism per day per kgm. (kilocals)	78.4	101.8	77
Average fasting catabolism per day per kgm.$^{3/4}$ (kilocals)	64.2	73.7	87
Average fasting catabolism per day per kgm.$^{2/3}$ (surface) (kilocals)	59.9	66.2	90
Period II: After cessation of injections			
Number of determinations	63	61	
Average body weight during respiration trials (grams)	391	282	139
Average fasting catabolism per day per rat (kilocals)	28.6	27.8	103
Average fasting catabolism per day per kgm. (kilocals)	70.0	95.2	74
Average fasting catabolism per day per kgm.$^{3/4}$ (kilocals)	56.0	69.9	80
Average fasting catabolism per day per kgm.$^{2/3}$ (surface) (kilocals)	51.9	63.1	82

rates of the injected rats amounted to only 90 per cent of that of the controls. This result confirms the earlier measurements of Lee, Teel and Gagnon who found that the metabolic rate per unit body surface area of their growth hormone rats was only 82 per cent of the corresponding rate for their controls.

A series of respiration trials was carried on in a period during which the injections had been stopped in one group of rats but continued in another. The results of this series are not included in the summary in table 4. The

[5] Fasting catabolism calculated from the O_2 consumption: 1 liter O_2 = 4.7 kilocalories.

results of 63 respiration trials on injected and 61 on control rats are presented under period II in table 4. These results were all obtained after the cessation of the injections.

The average body weight of the control rats is higher than in the first period, 282 grams as compared to 275. The body weight of the injected rats, on the contrary, is considerably decreased, 391 grams as compared to 452 grams in the first period. The average weight of the injected rats is therefore only 39 per cent larger than that of the controls. The metabolic rate per animal of the injected rats, however, is only 3 per cent larger than that of the controls, namely, 27.8 kilocalories per day in the second period as compared with 28.0 kilocalories per day in the first period. The metabolic level per unit of the $\frac{3}{4}$ power of body weight in the second period is

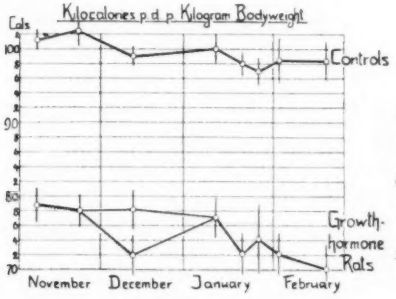

Fig. 1

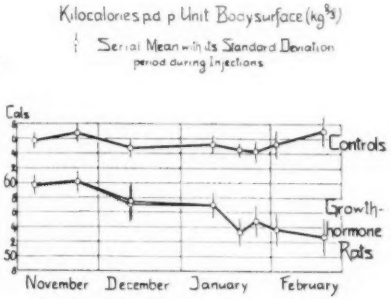

Fig. 2

Figs. 1 and 2. Each circle represents the average result for one series of experiments conducted on all the injected and control rats still living at the time. The length of the perpendicular lines at each of these marks represents the standard deviation of the result. The dotted line, which connects 3 results for the injected rats, indicates that the rats whose results are represented by these lines were still being injected.

69.9 kilocalories, which is slightly below the interspecific mean mentioned above.

The metabolic rate per unit surface of the injected rats in the second period is only 82 per cent of that of the controls. Our experiments therefore confirm the observation of Lee, Teel and Gagnon (1929) who observed a lowering of the metabolic rate during injection of growth hormone but they do not confirm the further observation of these authors that the metabolic rate of injected rats returned from a low to a normal level 3 weeks after the injections had been stopped.

Figure 1 illustrates the behavior of the metabolic rate per kilogram body weight of injected rats and controls during the course of time.

The figure shows that the metabolic rate per unit weight of the injected

rats is consistently lower than that of the corresponding controls and that the difference is highly significant throughout.

Figure 2 illustrates that even the metabolic rate per unit surface (kgm.$^{2/3}$) is consistently lower for the injected rats than for the controls. The differences are statistically significant.

If each result on the metabolic rate per unit surface (kgm.$^{2/3}$) of an injected rat is expressed as the percentage of the corresponding figure for the control littermate, then one obtains from 123 such comparisons for the metabolic rate of the injected rats an average of 87.05 ± 0.98 per cent of the metabolic rate of the controls.

d. Effect of injection on tissue respiration in vitro. The Warburg quotient Q_{O_2} for the diaphragms of our rats, i.e., the rate of oxygen consumption in cubic millimeters of oxygen per hour per milligram dry weight of tissue, is given in the first column of table 5. The Q_{O_2} of 6.1 mm.^{3}/hr. mgm. for the

TABLE 5

Oxygen consumption of diaphragm in vitro

In vitro: Average of 15 results on injected rats and 14 results on controls.
In vivo: Average of 120 results on injected rats and 116 results on controls.

	Q_{O_2} IN VITRO MM.^{3} O_2 P.H.P. MGM. DRY DIAPHRAGM	"Q_{O_2}" IN VIVO LITERS O_2 P.H.P. KGM. DRY RAT BODY	$\frac{Q_{O_2}}{"Q_{O_2}"}$
Injected rats	4.8 ± 0.4	1.6	3.0
Controls	6.1 ± 0.4	2.3	2.6
Injected in per cent of controls	79%	70%	115%
Difference: Controls—Injected	1.3 ± 0.56		
Random probability of difference	P = 3%		

surviving diaphragm of the controls is in very close agreement with the result obtained on rat diaphragm by Meyerhof, Lohmann, and Meier (1925) (6.0 mm.^{3}/hr. mgm.).

The rate of O_2 consumption of the diaphragms of the injected rats was on the average only 4.8 mm.^{3} per hour per milligram dry matter. The difference between the Q_{O_2} of the diaphragms from injected and control rats, namely, 1.3 ± 0.56, has a random probability of 3 per cent and may thus be regarded as statistically significant. If the Q_{O_2} of the diaphragm of each injected rat is expressed in the percentage of that of the corresponding control, then the standard deviation of one comparison is ± 20 per cent, and the mean of all 15 comparisons is 79.3 ± 5.2 per cent. The percentage difference in the Q_{O_2} between injected and control rat diaphragms *in vitro* (20.7 ± 5.2 per cent) is thus statistically highly significant.

It is shown in table 5 that the metabolic rate per kilogram dry matter of the living rat is 1.6 liters O_2 per hour for the injected rats and 2.3 liters per hour for the control rats. This "Q_{O_2} *in vivo*" of the injected rats is 70 per cent of that of the controls. A large part of the difference between the metabolic rates *in vivo* (30 per cent) is thus still observed in the metabolism *in vitro* (21 per cent). The question arose whether or not the lower rate of oxygen consumption in vitro of the diaphragms from the giant (injected) rats might be a result of a greater thickness of these diaphragms. The thickness was measured after the respiration trials by outlining the pieces taken out from the Warburg vessel on paper, cutting out the resulting figures, weighing them, and thus determining their area. The quotient of the weight of the pieces and their area is their thickness if the specific gravity is assumed to be unity. The thickness of the diaphragms of the injected rats was on the average 0.619 ± 0.034 mm., that of the control diaphragms 0.534 ± 0.039 mm. This is by no means a true measure for the thickness of the diaphragms in the rats' bodies since it is possible that the pieces shrank in the Ringer solution.

Using Warburg's formula for the maximum permissible thickness of tissue slices (Dixon, 1934), we find that none of our diaphragm pieces was too thick provided that we had pure oxygen in the Warburg vessels. This was probably the case, but we have no proof for it. To be sure that the thickness of the diaphragms did not influence our results, we should also, of course, be sure that Krogh's diffusion constant is not too high for the diaphragms in our experiments.

It is very likely that the transfer of gases in these slices that are shaken violently in the Warburg vessels is rather greater than the diffusion measured through a piece of fixed tissue. Furthermore, the constant given in this formula (1.4×10^{-5} cc./cm.^{2} cm. min.) is the value found by Krogh (1919) for muscular tissue at 20°C.; for 37° it would be approximately 17 per cent higher. This tends to increase our margin of safety. On the other hand, it is possible that a relatively larger amount of connective tissue in the diaphragms of the injected rats may reduce the rate of diffusion (Krogh found a rate of only 1.1×10^{-5} cc./cm.^{2}cm. min. for connective tissue).

Discussion. The metabolic rate per unit body weight of rats made giant by chronic injections of growth hormone was significantly smaller than the metabolic rate per unit body weight of their littermate controls which had normal size. The giant rats retained their lower metabolic rate per unit weight several weeks after the cessation of the injections. The metabolic rate per unit weight is thus not a genetically fixed constant but can be changed by somatic influences and such changes may persist for two months or more.

The metabolic rate in vitro per unit dry weight of the diaphragm of the giant rats was also lower than the corresponding rate for the diaphragms of

the controls. We do not, like other authors such as Busse, conclude from our results that we can explain the differences in the metabolic rates *in vivo* by the differences *in vitro*. We think, on the contrary, that the differences *in vitro* call for an explanation by the differences *in vivo*, which may not be completely eliminated in the ordinary procedure of measuring tissue metabolism *in vitro*. We agree with Holmes (1937) that the metabolic rate is "presumably dependent upon some influence residing in the cells themselves," but we assume that some of these influences in the cells, such as the concentration of respiratory enzymes, are essentially affected by the condition of the animal as a whole.

The composition, particularly the water, fat, or protein content of the body, does not give a satisfactory basis for explaining the differences in the metabolic rate of growth hormone rats and controls.

We have rather good indication that the post injectional loss in weight of the injected rats is mainly a loss of water; yet the metabolic rate per rat during the post injectional period was only 86 per cent[6] of that rate during the period of injections. By a reasoning similar to that applied to body fat in geese by Benedict and Lee (1937) this drop in metabolic rate per rat which accompanied a loss of water would lead to the conclusion that water was an "energy demanding" or "metabolically active" substance. Instead of using such paradoxes one may assume that a higher water percentage is simply an index for a higher percentage of metabolically active tissue. This assumption, however, would lead to the prediction of a higher metabolic rate per unit weight for the growth hormone rats during injections compared with that of the controls which would be contrary to the observations of Lee, Teel and Gagnon as well as those reported here.

Rather than assuming fixed metabolic rates for body constituents, such as the cells, we assume that the metabolic intensity of given cells varies according to their environmental condition. Cells that could not adapt their metabolic rate to the condition of the animal of which they are a part would either be unable to develop or if they did develop might endanger the existence of the animal as a whole.

The hypogony of the thyroid and the adrenal glands in the injected rats suggests the idea that the relatively low metabolic rate of our giant rats may be related, in part at least, to a relatively decreased production of thyroxin, cortin, and possibly adrenalin. Since a small gland does not necessarily secrete less than a large one, it remains for further work to test this idea. The metabolic rate of homeotherms compared interspecifically is proportional to the $\frac{3}{4}$ power of body weight (Kleiber, 1932). If one compares the metabolic rates of giant rats and their controls on this basis, then one is led to the conclusion that the metabolic rate of the giant rats

[6] This figure is an average of percentages each calculated for a single rat. The averages of the metabolic rates of all rats in table 4 lead to 81 per cent.

is abnormally low, and if one further assumes that the metabolic rate reflects the activity of the thyroid (and possibly the adrenal) glands, then one can conclude from our experiments that chronic injections of a pituitary extract designated as "growth hormone" decreased the activity of these endocrine glands to a greater extent than they increased body size and that this relative decrease of endocrine activity with respect to body size remains after the cessation of injections. Our experiment does not prove that body size, as such, controls the rate of metabolism, but it indicates that a somatic condition of the animal as a whole has a decisive influence on the metabolic rate and that this somatic condition can be correlated with body size.

SUMMARY

Daily injections into 22 female rats of alkaline extract of beef anterior pituitary glands (Evans' growth hormone preparation) over a period of 6 months stimulated growth. This growth was slightly hypergonic for the skin and slightly hypogonic for the liver. It was significantly hypogonic for the heart.

The thyroid glands of the injected rats were relatively smaller and their adrenal glands absolutely smaller than the corresponding glands of the controls.

The fasting catabolism of the rats was measured in respiration trials of 4 hours' duration during the period of injections as well as after the cessation of the injections (127 trials on injected rats and 118 on controls).

The metabolic rate of the injected rats per unit weight and even per unit surface was consistently and significantly lower than the corresponding rate of the controls. This result was observed during the period of injections as well as after the cessation of the injections. These differences in the metabolic rates per unit weight of giant rats and normal littermates can evidently not be explained by genetic differences. They can neither be explained by changes in the water or protein content of the rats.

The rate of oxygen consumption per unit of dry weight of tissue in vitro was smaller in the diaphragms of the injected rats than in the control diaphragms. This difference can obviously not be related to genetic differences of the tissues; it indicates that the respiration of tissues in vitro is still affected by the previous condition of the animal.

We regard our result as an example of somatic adaptation of tissue metabolism in vivo as well as in vitro to a condition of the animal as a whole, particularly to its endocrine system, which is in correlation to body size.

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THE ABSENCE OF ANAEROBIC RECOVERY IN MAMMALIAN MUSCLE

JACOB SACKS

From the Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor

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It is generally considered that the breakdown of adenosine triphosphate takes place early in the series of reactions which furnish the energy for muscular contraction. The adenylic acid formed by this reaction is held to be resynthesized to the original substance by transfer of phosphate groups from phosphocreatine. The phosphocreatine, in turn, is resynthesized by the energy liberated in the formation of lactic acid. These later processes are considered to be involved in the mechanism of anaerobic recovery. The basis for this formulation is to be found in the experiments of Embden et al. (1926) and Nachmansohn (1928) on frog muscle tetanized under anaerobic conditions. Embden and his co-workers found that some lactic acid formation takes place in the period immediately following a tetanus; Nachmansohn showed that in the early part of this post-stimulation period there was resynthesis of some 30 per cent of the phosphocreatine which had undergone hydrolysis during the tetanus. Although direct evidence for the interaction between adenylic acid and phosphocreatine in the intact muscle is lacking, it has been found to take place in muscle extract under certain conditions (Lohmann, 1934).

Investigations on contracting mammalian muscle (Sacks and Sacks, 1933, 1935) have led to a different formulation for the anaerobic phase. In these studies, phosphocreatine hydrolysis was found to accompany the formation of lactic acid, and the resynthesis of the former compound took place when lactic acid was removed from the muscle, by diffusion into the blood stream. The interpretation placed on these observations was that the formation of lactic acid supplied energy for contraction under anaerobic conditions, and that the hydrolysis of phosphocreatine furnished alkali to neutralize the lactic acid.

With regard to adenosine triphosphate, it is well established that in a fairly long tetanus or series of single twitches of frog muscle under anaerobic conditions, there is no net change in the amount of this substance present. Lohmann states that this is due to the extreme rapidity of the reconversion of adenylic acid to adenosine triphosphate. However, it has been shown

for mammalian muscle that, when it is possible to obtain breakdown of the triphosphate, the rate of resynthesis is quite slow (Sacks, 1938).

These experiments on mammalian muscle were all performed under conditions of normal circulation. Since there was a possibility that the results so obtained might not apply to the anaerobic conditions of the experiments on frog muscles, the work was repeated under conditions which were as anaerobic as is possible to maintain in mammalian muscle without loss of irritability. These conditions were obtained by ligating the blood vessels supplying the muscle while leaving it in place, and carrying out the stimulation within a few minutes after shutting off the circulation. The gastrocnemius muscles of cats under pentobarbital anaesthesia were used. The two muscles were prepared as in the previous studies. Then, in one leg all the vessels with branches to the gastrocnemius were tied off, except the popliteal artery and vein. These vessels, which constitute the principal supply, were left free until all other preparations for stimulation were complete. The muscle was attached to the isometric lever, the popliteal vessels then ligated, and the stimulation begun within one minute after the ligation. The muscle was frozen immediately at the end of the stimulation period, or, in the case of those allowed to "recover," 60 seconds after the end of the stimulation period. In this latter case, the attachments to the isometric lever were cut away simultaneously with the cessation of stimulation; the muscle was therefore in the relaxed state throughout the recovery period. The resting muscle, which had suffered no interruption of the circulation, was then frozen. The entire elapsed time from the ligation of the popliteal vessels to the freezing of the resting muscle, was less than five minutes. Under the conditions described, the traces of oxygen present at the beginning of the stimulation period must have disappeared very soon and the major portion of the contraction period and the entire recovery were completely anaerobic. The tension initially developed was as great as is seen in this muscle with normal circulation, but it began to fall within 5 seconds. After 15 seconds the tension had decreased to less than one-third of the initial value, and by the end of 60 seconds it had fallen to less than one-fifth of the maximum. In contrast, this muscle with normal circulation, stimulated in the same way, maintains the initial tension for about 30 seconds; over the next 30 seconds of stimulation there is a fall to about one-half of the maximum. The muscles were analyzed for the various phosphorus fractions and lactic acid by the methods previously described (1933).

It can be seen from table 1 that in the 60 seconds immediately following a tetanus of 15 seconds' duration, there is no resynthesis of adenosine triphosphate or phosphocreatine, nor is there any lactic acid formation. In other words, these reactions, which have been observed or postulated in frog muscle under similar conditions, and which are regarded as funda-

mental recovery processes, are lacking in mammalian muscle. The failure to accomplish these reactions is not due to exhaustion of the capacity to form lactic acid, for a longer period of tetanus results in an increased lactic acid content within the muscle. Nor can the failure to resynthesize the adenosine triphosphate be ascribed to lack of phosphocreatine, for the

TABLE 1

Changes in phosphocreatine, adenosine triphosphate and lactic acid in anaerobic tetanus of cat gastrocnemius muscle

Values expressed as milligrams per cent of P and of lactic acid

	PHOSPHOCREATINE HYDROLYZED	ADENOSINE TRIPHOSPHATE HYDROLYZED	LACTIC ACID FORMED
a. 15 seconds of anaerobic tetanus			
	22	7	142
	11	8	158
	10	6	93
	21	8	106
	11	7	138
Average	15*	7	127
b. 15 seconds tetanus, 60 seconds post-stimulation period			
	19	3	99
	17	5	148
	1	6	117
	24	5	152
	8	8	124
Average	14	5	128
c. 60 seconds anaerobic tetanus			
	7	9	168
	19	3	168
	12	9	136
	10	4	162
	9	6	161
Average	11*	6	159

* On account of the wide variations in the individual values making up these averages, the difference does not necessarily have any significance.

amount of this substance present at the end of 15 seconds of anaerobic tetanus is about half of that in the resting muscle.

It must be pointed out that the views generally held concerning the anaerobic recovery mechanism do not regard it as being capable of bringing about more than a partial recovery. However, this will not solve the

difficulty, for if it is held that the lactic acid formed represents the resynthesis of a thermally equivalent amount of phosphocreatine, and this in turn the resynthesis of an equivalent amount of adenosine triphosphate, then these data would require that the anaerobic recovery process be completed during the contraction period itself. Also, since the heat of formation of 2 mgm. of lactic acid from glycogen is equivalent to that required to resynthesize 1 mgm. of phosphocreatine-P, such a formulation would require the muscle after 15 seconds of tetanus to have recovered to the extent of 80 per cent. The anaerobic process in frog muscle is considered to be able to bring about recovery to the extent of only 30 per cent. Furthermore, this interpretation would require that the more nearly fatigue is approached, the more complete the recovery.

Under the circumstances, it is difficult to avoid the conclusion that mammalian muscle does not undergo anaerobic recovery in the sense that the term has been applied to frog muscle. The formation of lactic acid must then be considered as a source of energy for activity under anaerobic conditions, rather than as a recovery mechanism.

SUMMARY AND CONCLUSIONS

1. In the period immediately following a tetanus of mammalian muscle under anaerobic conditions, there is no evidence that resynthesis of adenosine triphosphate or phosphocreatine takes place, nor is any lactic acid formed.

2. The failure to accomplish these postulated "recovery" reactions is not due either to inability to form more lactic acid or to deficiency of phosphocreatine.

3. Mammalian muscle does not show the phenomena of anaerobic recovery which have been described for frog muscle.

4. The formation of lactic acid in muscular contraction under anaerobic conditions is to be regarded as a means of supplying energy for contraction, rather than as a recovery process.

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EVIDENCE FOR ADRENALINE IN ADRENERGIC NEURONES

W. B. CANNON AND K. LISSÁK[1]

From the Department of Physiology in the Harvard Medical School

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Neuroblasts migrating outward from the primitive axis of spinal cord segments develop mainly into the ganglia of the sympathetic system; some of them, however, are transformed into the adrenal medulla. Thus most of these neuroblasts become sympathetic neurones; a small remnant become secreting cells which, on being stimulated, discharge adrenaline.[2] Nearly all sympathetic neurones, likewise, when they are stimulated, discharge at their endings a substance which, theoretically, has been assumed to be adrenaline. This substance is so altered in some effector organs, however, that when circulating later in the blood stream it may have, on certain sympathetically innervated structures, effects so different from the effects of adrenaline as to warrant the specific name, sympathin (see Cannon and Rosenblueth, 1937, pp. 88, 98). This further change does not modify the evidence that the neuroblasts which develop into sympathetic neurones may properly be regarded as becoming cells which secrete, quite as truly as the corresponding neuroblasts which develop into the cells of the medulla of the adrenal gland. Since adrenaline is the characteristic constituent found when extracts are made of the medullary cells, and since sympathetic neurones are like the medullary cells in origin and in producing adrenaline, or an adrenaline-like substance, the question arose as to whether extracts of these neurones might not yield adrenaline. The present research was directed toward obtaining further evidence regarding that possibility.

METHODS. Extracts were made of mesenteric nerves as postganglionic sympathetic fibers, either alone or with sympathetic ganglia; also extracts of other nerves, and of organs containing sympathetic nerve endings, the heart and liver especially, as well as the uterus, intestinal muscle and

[1] Rockefeller Fellow from Hungary.

[2] For many years investigators in this laboratory have used the term "adrenin" and more recently "adrenine" to designate the secretion and extracts of the adrenal medulla. This was done with respect to the suggestion of Schafer, who, with Oliver, first observed the vasopressor action of preparations of the adrenals. Few others have adopted this usage. Meanwhile, in foreign languages, and commonly in English writing, the term "adrenaline" has become customary. In accordance with this growing practice that word has been adopted here.

arteries. The procedure followed in preparing nervous tissue for the tests was that described by Lissák (1939). When an organ was used it was washed in salt solution until free of blood, dried, weighed, minced and ground in sea sand, then mixed with bicarbonate-free Ringer's solution (2 to 3 cc. per gram of tissues). This was the method for the heart, uterus, intestinal muscle and arteries (brachial, cervical, femoral and mesenteric). The liver was treated differently in order to separate the vessels from the parenchyma. The whole organ was first softened by gentle pounding, and thereafter the pulp was carefully scraped from the blood vessels; doubtless in this process some of the finer vascular endings were torn away so that the pulp was not wholly free of gross vascular elements. The mixture of finely divided tissue in Ringer's solution was dialyzed against an equal volume of Ringer's. Thereupon the dialysate was brought to about 1/100 N HCl and desiccated in vacuo. When to be tested, the dry residue was dissolved in 1 to 2 cc. of distilled water. Obviously the salts in this small volume would be concentrated. Control experiments, however, made by drying 30 cc. of bicarbonate-free Ringer's solution brought to about 1/100 N HCl, dissolving the residue in 2 cc. of distilled water and using it for various tests, showed that the concentrated salts had, by themselves, no effect.

Since there is evidence that cocaine inhibits the oxidation of adrenaline (cf. Luco and Lissák, 1938), that drug was commonly injected, intravenously (8 mgm. per kgm. body weight), about ten minutes before the heart or the liver was removed.

The tissues to be tested were taken mainly from cats, though the frog heart, and sympathetic nerves of cattle, sheep and dogs also were used. Almost all the tests were made on the cat, usually under dial anesthesia (Ciba, 0.7 to 0.8 cc. per kgm., intraperitoneally). The adrenal glands were invariably tied off. To render the reacting structures more sensitive cocaine (8 mgm. per kgm.) was commonly injected, into the femoral vein, at the start of the experiment. In addition, the hypodynamic frog heart was employed for testing nearly every extract in order to obtain confirmatory evidence (see table 1).

Results. A. *The effects on blood pressure.* For blood-pressure tests either the preparation described by Elliott (1912) was used (i.e., the cat pithed to the mid-thorax), or an animal under dial anesthesia, with both common carotids tied and the vagi cut in the neck. In either case an extract of cat's mesenteric nerves, injected into the femoral vein, has the typical adrenaline-like vasopressor action (fig. 1*a*). A similar positive effect has been recorded from extracts of mesenteric nerves taken from sheep and dog. Evidence that this does not result from some incidental agent, associated with the preparatory process, is found in absence of the rise when an extract of cholinergic nerves (the vagi), made by the same process, is tested (fig. 1*b*).

That an organ enclosing sympathetic nerve endings can yield a substance having the same effect as adrenaline is proved by injections of extracts of the heart from either the frog or the cat. In figure 2 are registered the increases of blood pressure caused by an extract of normal cat

TABLE 1

Adrenaline-like action of tissue extracts on sympathetic indicators

A + mark indicates an effect like that of adrenaline, − indicates an opposite effect, and 0 no change.

TISSUES EXTRACTED	INDICATORS				
	Frog heart	Blood pressure	Iris	Nictitating membrane	Non-pregnant uterus
Nerves: Adrenergic	+	+	+	+	+
Cholinergic	0	0	0	0	0
Cat heart: Normal	+	+	+	+	+
Sympathectomized	0	0	0	+*	0
Frog heart	+	+	+	+	+
Liver vessels: Normal	+	+	+	+	−
Sympathectomized		−	0	0	
Liver pulp: Normal	−	0−	0	0	−
Sympathectomized		−	0	0	

* Slight +.

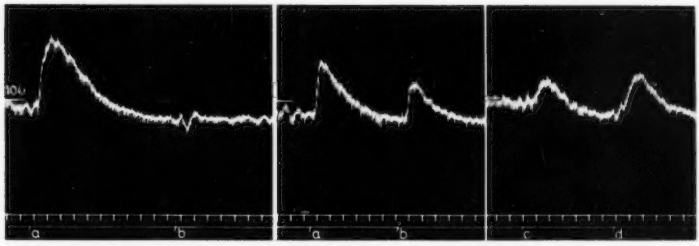

Fig. 1

Fig. 2

Fig. 1. Blood pressure. Cat. Dial. Adrenals removed. Carotids tied. Vagi cut. At *a*, injection of an extract of mesenteric nerve fibers; *b*, an extract of cervical vagal trunks from the cat. Time line, half-minutes; zero blood pressure.

Fig. 2. Same cat as in figure 1. At *a*, injection of ¼ of an extract of a 15-gram heart removed after cocaine; *b*, ¼ of an extract of a 16-gram cat heart removed without preliminary cocaine; *c*, adrenaline (0.25γ); *d*, extract of frog hearts.

heart removed after cocaine, *a*, and the smaller effect of the extract from a somewhat larger heart removed without a preliminary cocaine injection, *b*. This result, confirmed in other instances, is in accord with the testimony of Luco and Lissák (1938) that cocainization favors the appearance of

sympathin, which is assumed to be dependent on a discharge of adrenaline at the nerve terminals. Figure 2, *c* and *d*, shows the effects of injection of adrenaline (0.25γ) and of an extract of normal frog hearts. When extracts were allowed to stand they turned a yellow-brownish hue; then, a frog heart extract, for example, instead of causing a rise, produced a slight lowering of blood pressure.

Since the normal heart yields a substance acting like adrenaline on blood pressure, an effect such as is produced by sympathetic nerves, the question arose as to whether the same effect would result from a heart in which the sympathetic fibers had degenerated. For an answer the thoracic sympa-

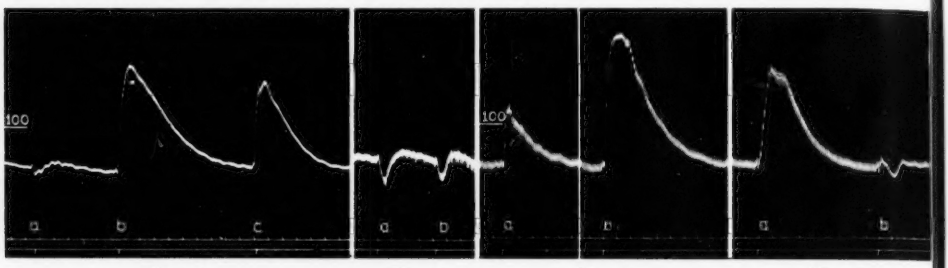

Fig. 3 Fig. 4 Fig. 5 Fig. 6

Fig. 3. Blood pressure. Cat, pithed through orbit to mid-thorax. At *a*, injection of ¼ of an extract of a 13-gram cat heart with sympathetics degenerated. At *b*, ¼ of an extract of a 13-gram cat heart, normal. At *c*, 1 cc. Ringer's solution containing 2γ adrenaline.

Fig. 4. Blood pressure. Cat, pithed through orbit to mid-thorax. After ergotoxine (65 mgm.), adrenaline (2γ) injected at *a*, and an extract of mesenteric nerves (¼ of 670 mgm.) at *b*.

Fig. 5. Blood pressure. Cat, pithed through orbit to mid-thorax. At *a*, injection of ¼ of an extract of a 15-gram heart; *b*, same injection as before. Between *a* and *b*, cocaine hydrochloride (8 mgm. per kgm.) injected.

Fig. 6. Blood pressure. Cat, pithed through orbit to mid-thorax. At *a*, injection of ¼ of an extract of liver arteries (6 grams); *b*, injection of ¼ of an extract of liver pulp (12 grams).

thetic chains on both sides from the stellate ganglia to the sixth or seventh rib, and the lower cervical ganglia, were extirpated. At least four days were allowed for degeneration to occur. As shown in figure 3*a*, the extract from the heart thus treated has no positive action on blood pressure.

Typical of the blood-pressure response to adrenaline, in the cat, is a reversal by ergotoxine. Figure 4 presents a record showing the characteristic drop of pressure when, after ergotoxine, adrenaline and an extract of postganglionic sympathetic fibers were injected. The same phenomenon occurred when extracts of heart and liver vessels were tested.

Also typical of the blood-pressure response to adrenaline is a sensitization

by cocaine. In figure 5 are shown the increases of pressure produced by two equal injections of an extract of normal cat heart; between *a* and *b* 19 mgm. of cocaine hydrochloride (8 mgm. per kgm.) were injected.

The Elliott preparation is a reliable means of quantifying the adrenaline content of an unknown solution. When the increase of blood pressure produced by extracts of mesenteric nerves and cat heart was matched with that produced by adrenaline it was found, in two cases, that the nerves contained 5 and 5.8γ per gram and the cat hearts 0.5 and 0.8γ per gram, respectively. The assays for the nerves fall within the range found by Lissák (1939) in tests on the frog heart.

An extract of a gluteal muscle, prepared as cardiac muscle was prepared, had no noteworthy effect on the blood-pressure record. And, as might be surmised from this result, a control injection of the concentrated Ringer's solution (see p. 766) was without influence.

A question of considerable theoretical importance is whether sympathetic fibers are distributed to parenchymal cells of the liver or only to its blood vessels. With due regard to the difficulty of obtaining pure hepatic pulp, already mentioned, it appears that the distribution of the fibers is chiefly, if not wholly, to the vessels. Thus, in three instances, the injection of an extract of the vessels from a normal liver caused, after a brief and minor initial drop of blood pressure, a high rise (e.g., 58 mm. Hg), and in a fourth instance a pure rise of 80 mm. Hg (see fig. 6*a*). An extract of pulp, on the other hand, either produced a pure fall or a slight fall and a slight rise (e.g., 14 mm.) or had an insignificant action (fig. 6*b*).

If the hepatic nerves are cut and allowed to degenerate, both the vascular and the parenchymal extracts produce only a fall of pressure. This phenomenon emphasizes the point, indicated by the mixed effects of liver extracts just described, that the adrenaline-like agent is doubtless not alone in passing through the dialyzing membrane; other substances are present, causing a fall of blood pressure (cf. Vincent and Sheen, 1903; Miller and Miller, 1911) and therefore working in opposition to a vasopressor agent. Extracts of nerves and heart (though having material which masked an adrenaline spectrum) appeared to contain only a small amount of a hypotensive factor, as shown by absence of a pressure drop when an extract of cholinergic nerves (fig. 1*b*) or heart muscle, free of sympathetic fibers (see fig. 3*a*), was injected; on the other hand, a dialysate from the non-pregnant cat uterus had mixed effects like liver vessels, and a dialysate from intestinal muscle (separated from the mucosa) induced a straight drop of arterial pressure. These preparations of smooth muscle were peculiarly productive of hypotensive effects.

B. *The effects on the iris.* The well-known sensitiveness of the iris to circulating or instilled adrenaline was increased by removal of the superior cervical ganglion about a week before the tests and by injection of cocaine

when the animal was made ready for the acute experiment. The extracts were introduced through a fine needle, usually in 0.5 cc. quantities, into the carotid artery on the sensitized side. Two powerful lights illuminated the field to be photographed and reflexly narrowed the pupil when the iris was not dominated by a chemical agent. A photograph was taken in each instance as soon as a maximal state was reached after an injection—an interval of a few seconds. Before each injection the "normal" condition of the iris was photographically registered.

In a series of photographs, some of which are reproduced in figure 7, the "normals" were so nearly the same in all cases that only one is presented, *a*. Inspection of figure 7 reveals at once that the extracts of mesenteric nerves, *b*, and of normal cat heart, *d*, had, like 0.5γ of adrenaline, a maximal effect. But also the concentrated Ringer's solution (see p. 766) caused some widening of the pupil—about as much as the extract of the vagus nerves, *c*. It appears that the iris is not precisely specific in its reactions. The slightly positive effect, therefore, produced by the extract of the heart of which the sympathetic fibers had been cut and allowed to degenerate, *e*, may not be indicative of adrenaline from the continued presence of sympathetic endings, but may result from some other dialysate or from concentrated salts. The much greater dilatation of the pupil induced by extracts of known sympathetic sources, *b* and *d*, as contrasted with those from a source non-sympathetic or questionable, *c* and *e*, is sufficiently striking, however, to offer good evidence in support of that already given by the blood-pressure experiments.

A question of special importance, as will be emphasized in the discussion, is the type of effect of liver extracts on the iris—whether like sympathin, negative, or like adrenaline, a retraction with enlargement of the pupil. As shown in figure 7, *i* and *j*, an extract of liver vessels (4 grams) evokes an extreme pupillary dilatation, whereas an extract of liver pulp (10 grams) has only a slight dilator action.

C. *The effects on the nictitating membrane.* In figure 8 are shown contractions of the nictitating membrane produced by adrenaline, and by extracts of normal cat and frog hearts and mesenteric nerves injected into the right femoral vein. On the other hand, an extract of vagus nerves had no effect (fig. 8*d*).

The defect of the nictitating membrane as an indicator is a lack of specificity in its sensitiveness (cf. Rosenblueth, 1932*a*). It contracts to some degree in response to an extract of cat heart in which the sympathetic fibers have degenerated. This result is not in accord with the absence of any noteworthy influence of that extract on blood pressure (see fig. 3*a*). The record in figure 8*b*, therefore, may reasonably be regarded as in part due to some other dialysate than adrenaline.

D. *The effects on the non-pregnant uterus of the cat.* The typical relaxing

action of adrenaline on this organ (recording as described by Cannon and Rosenblueth, 1933) is illustrated in figure 8*a*. Extracts of normal cat heart (fig. 8*b*) and frog heart (fig. 8*c*), and cat blood vessels, as well as

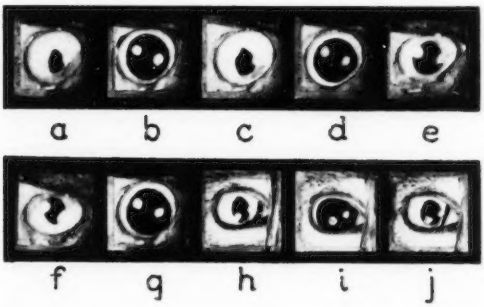

Fig. 7. Photographs of cat's iris, sensitized by denervation and cocaine. Nictitating membrane removed. Injections (0.5 cc.) into ipsilateral carotid artery: *a*, "normal" condition (control); *b*, extract of mesenteric nerves (i.e., $\frac{1}{4}$ of 850 mgm.); *c*, vagus nerves ($\frac{1}{4}$ of 370 mgm.); *d*, normal cat heart ($\frac{1}{5}$ of extract of a 17-gram heart); *e*, sympathetically denervated cat heart ($\frac{1}{5}$ of a 19-gram heart); *f*, Ringer's solution, concentrated 5-fold; *g*, adrenaline, 0.5γ; *h*, normal control in another cat; *i*, extract of liver vessels ($\frac{1}{2}$ of 4 grams); *j*, liver pulp ($\frac{1}{4}$ of 10 grams).

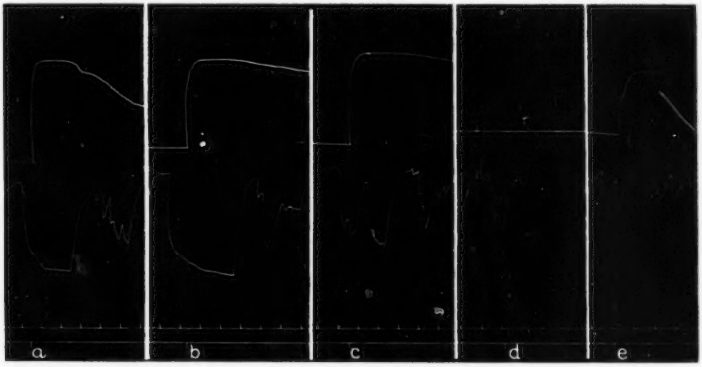

Fig. 8. Cat. Dial. Left superior cervical ganglion removed 10 days before. Upper record, left nictitating membrane; lower, right horn of nonpregnant uterus. Injections (1 cc.) into right femoral vein: at *a*, 2γ of adrenaline; *b*, extract of normal cat heart ($\frac{1}{4}$ of a 15-gram heart); *c*, cat mesenteric nerves ($\frac{1}{2}$ of 500 mgm.); *d*, cat vagus nerves ($\frac{1}{2}$ of 350 mgm.); *e*, normal frog hearts ($\frac{1}{4}$ of 11 grams).

extracts of mesenteric nerves (fig. 8*c*), had the same effect. When similar preparations of the vagus nerves (fig. 8*d*) or of a gluteal muscle were injected, no change was produced.

The uterus varies considerably from animal to animal in its sensitiveness. In order to have more definite proof of the action of sympathetic nerve extracts on that organ the iliac arteries were tied in some animals (to prevent loss into the legs) and the injection was made into the lower abdominal aorta. The relaxing effects on a non-pregnant uterus when preparations of the normal heart, normal arteries and the mesenteric nerves of the cat were thus injected were often more striking than those registered in figure 8. Extracts of liver vessels and pulp, of intestinal muscle and the uterus itself, not only had no relaxing effect on the non-pregnant uterus, but actually a produced contraction. It will be recalled that these are the sources which yield extracts peculiar in exerting an action opposite to that of adrenaline on blood pressure. It appears that dialysates from these organs may partially or wholly overwhelm the effect produced by an

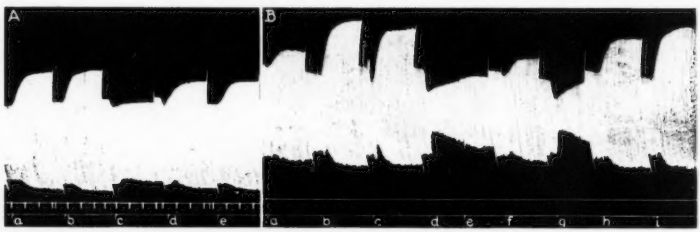

Fig. 9. Hypodynamic frog heart. A. At *a*, adrenaline 0.01γ; *b*, dialyzed extract of 15-gram normal cat heart in 25 cc. Ringer, diluted 8 times; *c*, dialyzed extract of 14-gram cat heart (denervated 11 days) in 25 cc. Ringer, diluted 8 times; *d*, adrenaline 0.005γ; *e*, dialyzed extract of 10 grams of frog hearts in 25 cc. Ringer, diluted 10 times. B. At *a*, adrenaline 0.001γ; *b*, adrenaline 0.01γ; *c*, dialyzed extract of cat liver vessels (10 grams in 20 cc. Ringer), diluted 5 times; *d*, dialyzed extract of cat liver pulp (10 grams in 20 cc. Ringer), diluted 10 times; *e*, atropine 1:100,000; *f*, same as *c*, 10-fold dilution; *g*, same as *d*; *h*, adrenaline 0.005γ; *i*, same as *c*.

adrenaline-like substance with which they may be associated. The typical relaxing action on the uterus of an extract from blood vessels as contrasted with the contractile action of other smooth-muscle extracts is puzzling and will require further investigation.

E. *The effects on the hypodynamic frog heart.* This highly sensitive indicator was employed not only to determine the influence of extracts of sympathetic nerves (cf. Lissák, 1939), but also the influence of extracts of other structures. Dialysates from striated muscle and from cat heart in which sympathetic fibers had degenerated had no positive action. On the other hand, dialysates from the normal cat heart and from frog hearts accelerated and strengthened the contractions. When preparations of the liver were tried, the pulp was found to be a depressant (not altered by

atropine) and the vessels caused typical adrenaline-like, positive chronotropic and inotropic effects (see fig. 9).

F. *Chemical evidence for adrenaline in sympathetic fibers.* Shaw (1938) has described a very delicate and specific chemical test for adrenaline—a blue color resulting from an adrenaline reduction of arsenomolybdic acid. Addition of alkali intensifies the color between 2 and 3.5 times, if the reducing agent is adrenaline. Applied to extracts of rabbit and frog hearts Shaw found adrenaline present and that the former contained much less than the latter.

When this method was tried on concentrated extracts of normal cat heart and cat's mesenteric nerves a blue color resulted, that was deepened by adding alkali. Concentrated extracts of cat vagus nerves and cat heart containing degenerated sympathetic fibers yielded a slightly bluish color, not altered by alkali.

DISCUSSION. *Previous observations.* In 1933, Bacq reported that extracts of the endocardium of the sheep and dog gave a marked positive reaction with the Viale test—a reaction which disappeared after exposure to air or oxygen and which was much less pronounced if the cardiac sympathetic fibers had degenerated. Whether the active substance was sympathin or adrenaline could not be determined because the Viale test is not discriminative; and unfortunately it is not specfic for adrenaline. The next year v. Euler (1934) announced that extracts of the prostate and related glands had adrenaline-like effects on blood pressure and intestinal movements, an observation which he explained by the presence of chromaffine cells in the structures examined. Loewi (1936) dialyzed extracts of frog heart and, by matching the dialysate with adrenaline as seen in the response of the hypodynamic heart and by finding the specific fluorescence described by Gaddum and Schild (1934), came to the conclusion that adrenaline was present. He (1937) also found it in the heart of the guinea pig. Shaw's evidence that the frog and rabbit heart contains adrenaline has already been mentioned. A footnote in a paper by Loewi and Hellauer (1938), that appeared while the present research was in progress, stated that their investigations of the adrenaline content of pre- and postganglionic fibers had been interrupted.

From the results detailed in the foregoing pages it is clear that the investigators mentioned above were probably concerned with adrenaline in the nerve fibers existing in the organs which they studied. At least, there is no evidence that sympathetic impulses assure an adrenaline content of cells innervated by sympathetic neurones. The simple explanation of the disappearance of the adrenaline effect after degeneration of the sympathetic supply to the heart and the liver, for example, is that the adrenaline of the nerve fibers vanishes as the degenerative process proceeds.

With Loewi's (1936) testimony that extracts of the frog heart contain

adrenaline the results here reported are in full agreement. Loewi's conclusion, however, that the substance liberated by sympathetic stimulation is adrenaline, must be judged after considering what is meant by "liberated." Sympathetic stimulation liberates from the liver a substance which does not cause dilatation of the pupil (Cannon and Uridil, 1921); but an extract of liver vessels, like adrenaline, causes a maximal dilatation (fig. 7 *i*). Excitation of sympathetic nerves in various parts of the body liberates a substance which, after ergotoxine, induces a rise of blood pressure (Cannon and Rosenblueth, 1933); but, after ergotoxine, an extract of these nerves, like adrenaline, elicits a pure fall (fig. 4*b*). There is evidence that the substance liberated by the heart when the accelerators are activated does not relax the non-pregnant uterus (Cannon and Rosenblueth, 1933); but extracts of the heart, like adrenaline, caused marked relaxation of that organ (fig. 8*b*). If "liberate" means to set free into the blood stream, evidently the material liberated by sympathetic impulses in these instances, as indicated by its effects, is not the same as adrenaline.

It is obvious that the differences between sympathin, circulating with the blood, and adrenaline, contained in sympathetic neurones, are demonstrated only when the reactions of various bodily organs are registered while in the body. The advantage of this basis for judgment Loewi did not have. His observations, as well as those of Bacq and Shaw, are in agreement with the results here reported, that the peculiar material which is extracted from sympathetic neurones is adrenaline itself. In 1932 (*b*), Rosenblueth, after studying the action of adrenaline and nerve impulses on smooth muscle and showing that the two exhibited the same characteristics, suggested that the chemical mediator, M, discharged at sympathetic endings, was probably adrenaline, A, and, like adrenaline, it united with a hypothetical substance, H, in the activated cell, making a combination MH or AH which evokes a response fixed by the amount formed. Later, basing their inferences on the facts mentioned in the paragraph above and on Langley's (1921) notion of a differential receptive substance in the cell, Cannon and Rosenblueth (1933) put forth the theory that the hypothetical H could be either excitatory, E, or inhibitory, I. In this view one of two kinds of sympathin would emerge from a structure receiving sympathetic impulses, sympathin E or sympathin I, dependent on whether the impulses induced excitation or inhibition. The results reported in the present communication are consistent with the view that sympathetic neurones liberate adrenaline at their terminals and that this agent, when it escapes into the blood stream, has been modified in such manner that it has the peculiar actions of sympathin on remote organs in the body. The designation, "adrenergic nerve fibers," would thus be quite exact.

The question might arise as to whether, in some instances, sympathin might be formed during the preparation of extracts of tissues containing

adrenergic fibers. Extracts of the liver, for example, and of the uterus and intestinal muscle, did not induce relaxation of the non-pregnant uterus, as did adrenaline and extracts of blood vessels, heart and adrenergic nerves. Was that because sympathin E was formed? The evidence appears to be opposed to that inference. Sympathin E is typically discharged into the circulation when the hepatic nerves are stimulated (Cannon and Rosenblueth, 1933). It causes rise of blood pressure, contraction of the nictitating membrane, no noteworthy dilatation of the pupil and no change in the non-pregnant uterus. Extracts of the liver vessels likewise raise blood pressure and retract the nictitating membrane, but, not likewise, they dilate the pupil widely (fig. 9 *i*) and induce shortening of the non-pregnant uterus. They do not, therefore, act like sympathin E. Sympathin I would, theoretically, induce relaxation of the non-pregnant uterus of the cat. This is the opposite of what is seen. Furthermore, when ergotoxine is given, liver extracts which previously increased blood pressure, evoke a drop. With lack of evidence for sympathin to account for the peculiar action of some tissue extracts, the presence of special dialysates from the liver and from uterus and intestine seems the most likely explanation of effects such as are not elicited by adrenaline.

An observation of noteworthy importance is the marked difference between the effect of extracts of liver pulp and liver blood vessels on the blood pressure (fig. 6), on the iris (fig. 7, *i* and *j*) and on the frog heart (fig. 9B, *c*, *d*). In consideration of the stupendous multitude of cells in the hepatic parenchyma, the relatively small nerve bundles accompanying the hepatic artery seem inadequate for their innervation. Riegele (1928) has, indeed, described a network of extremely delicate nerve filaments between the liver cells, with offshoots reaching into the cellular cytoplasm. The existence of this nervous "terminal reticulum," however, has been questioned by Nonidez (1937) who reports that the silver method used by Riegele may also impregnate fine connective tissue fibers, which show identical staining reactions. "Up to the present," Nonidez declares, "no nervous structure resembling a net work has been described in the liver." Physiological evidence is in accord with the view that the nerve supply of hepatic cells is slight or lacking, for in the absence of the adrenal glands the hepatic nerves have relatively little or no influence in evoking glycogenolysis (cf. Bulatao and Cannon, 1925; Britton, 1928). The sympathetic control of branches of the hepatic artery, however, is well known (the slight glycogenolytic action from stimulation of the hepatic nerves might be due to temporary relative asphyxia of the hepatic cells resulting from vasoconstriction or to local diffusion of sympathin from vascular muscles (see Bodo and Benaglia, 1938). Concordant with these suggestions is the failure of extracts of liver pulp to manifest the presence of sympathetic neurones, and the marked influence of extracts of liver blood

vessels. It should be noted, however, that the idea that liver cells have meager sympathetic innervation brings forth an inconsistency—the existence of an important organ in the body which adrenaline readily affects, though not acting then as a sympathomimetic agent.

The presence of a few chromaffine cells in sympathetic ganglia would confuse the evidence that adrenaline is present in adrenergic neurones, if the ganglia were used in making extracts. They were rarely used. And, furthermore, chromaffine cells have not been found associated with mesenteric nerves, heart muscle or liver vessels, extracts of which yielded adrenaline-like effects.

The evidence for adrenaline in adrenergic neurones of vertebrates is interestingly related to observations by Gaskell (1919) on the nervous system of annelids. He found in the ganglia of various species nerve cells which gave a chromaffine reaction. They sent fibers to contractile blood vessels which proved to be sensitive to adrenaline. Furthermore, extracts of these ganglia yielded a substance which had a physiological action typical of adrenaline (relaxation of the non-pregnant cat uterus). Gaskell suggests that these nerve cells are the common ancestors of the sympathetic ganglion cells and the cells of the adrenal medulla.

SUMMARY

Extracts of adrenergic fibers, whether from mesenteric nerves or from an organ containing such fibers (e.g., the heart), are like adrenaline in raising blood pressure (figs. 1, 2, 3, 4, 5, 6), in being sensitized by cocaine (fig. 5), in causing a fall of blood pressure after ergotoxine (fig. 4), in producing a dilation of the pupil (fig. 7), a contraction of the nictitating membrane (fig. 8) and a relaxation of the non-pregnant cat uterus (fig. 8), in making the frog heart beat more rapidly and more vigorously (fig. 9), and in responding to a specific chemical test for adrenaline (p. 773).

Though extracts of liver blood vessels may have mixed effects on arterial pressure an adrenaline-like pressor action predominates (fig. 6 *a*); an adrenaline-like action is also demonstrable on the iris (fig. 7 *i*) and on the nictitating membrane. Liver pulp (which doubtless contains some small vascular twigs) may produce a pure fall of pressure, have a slight mixed effect or be almost inactive (fig. 6 *b*). If cut hepatic nerves have degenerated extracts of the vessels or the pulp cause only a pressure fall.

Extracts of uterine and intestinal muscle have mixed or depressive effects on blood pressure, and, like extracts of liver vessels and pulp, have a contractile influence on the non-pregnant uterus. Mixed and anomalous actions of the extracts are attributed to peculiar dialysates from the tissues mentioned, that act oppositely to adrenaline.

Extracts of vagus nerves and skeletal muscle, and also of the heart in

which sympathetic fibers have degenerated, produce no distinctive adrenaline-like effects on blood pressure or iris (cf. figs. 3 and 7).

From these observations and those reported in the previous paper (Lissák, 1939) we infer that adrenaline is distributed throughout adrenergic neurones.

The relation of these results to the differences between adrenaline and sympathin are discussed. The results are consistent with the view that adrenaline is liberated at the endings of adrenergic fibers, and that sympathin, which escapes from the stimulated region into the blood stream, is this adrenaline modified in the affected cells.

The evidence that the parenchymal cells of the liver do not receive sympathetic fibers is considered in relation to the effect of adrenaline upon them.

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EFFECTS OF EXTRACTS OF ADRENERGIC FIBERS ON THE FROG HEART[1]

K. LISSÁK[2]

From the Department of Physiology in the Harvard Medical School

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The early observations on the effects of nerve extracts, made by Cleghorn (1899, 1900), were repeated and criticized by Halliburton (1901 a, b). A new interest in this field was awakened by the discovery of chemical mediations of nerve impulses. Numerous investigators began to study the acetylcholine content of different nerves and ganglia (Witanowski, 1925; Plattner, 1933; Chang and Gaddum, 1933; Binet and Minz, 1934; Kwiatkowski, 1935; Barsoum, 1935; Brown and Feldberg, 1936). From the results of experiments carried out in these investigations it appears clear that all motor, parasympathetic and preganglionic sympathetic nerves and also sympathetic ganglia contain acetylcholine in different amounts. Evidence has accumulated that nerve impulses delivered by these nerves discharge acetylcholine at their terminals. The postganglionic sympathetic fibers with few exceptions liberate an adrenaline-like substance at their endings. Dale (1933) proposed that fibers liberating acetylcholine be named cholinergic and that postganglionic sympathetic fibers liberating an adrenaline-like mediator be named adrenergic. Whether the postganglionic adrenergic fibers contain acetylcholine or adrenaline had not been determined. It was suggested by Doctor Cannon that I undertake a study of this question.

METHOD. Cats, rabbits and dogs anesthetized with dial (Ciba, 0.6 to 0.8 cc. per kgm., intraperitoneally) were used. The nerves to be examined were isolated and removed, washed in salt solution, dried with filter paper, then weighed on a torsion balance, afterwards minced and ground up with sea sand (Merck) in a mortar with bicarbonate-free Ringer's solution (about 1 cc. per 100 mgm. nerve). The extracts were dialyzed, through a cellophane membrane, against an equal amount of bicarbonate-free Ringer's solution, according to the method of Loewi (1936), for 3 hours in a shaking-machine. The dialysate was tested on isolated frog hearts, according to the method of Straub. When used for testing for positively acting substances, the hearts were rendered hypodynamic by frequent

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[2] Rockefeller Fellow from Hungary.

washing with Ringer's solution for 3 to 4 hours before the testing procedures were carried out. Such hearts are very sensitive to adrenaline; 0.001γ is easily detectable. If acetylcholine and the adrenaline-like substance are both present in an extract, and a "normal" (i.e., not hypodynamic) heart serves as the test object, the response is the negative effect; there is a decrease of amplitude and rate of the heart, typical of acetylcholine. For quantitative comparison titrations with acetylcholine and with adrenaline were made on the same heart.

The drugs used were: adrenalin (Parke, Davis), acetylcholine (Merck), ergotoxine ethanesulfonate (Burroughs, Wellcome), and physostigmine salicylate (Merck).

RESULTS. A. *Extracts of different nerves in Ringer's solution plus physostigmine.* Extracts were made from the vagus, cervical sympathetic, sciatic, phrenic, thoracic sympathetic chain, mesenteric plexus and the

TABLE 1

Acetylcholine equivalent of extracts in γ per gram nerve

Average values calculated from 10 experiments. The maximal deviation from the average was 30 per cent.

NERVES	CATS	DOGS	RABBITS
Cervical vagus	10	8	8
Cervical sympathetic trunk	11		6
Superior cervical ganglion	16		14
Sciatic	3.5	4	2.5
Phrenic	1.8	1.5	1.4
Superior mesenteric plexus	1.5	2	
Thoracic sympathetic chain (including the ganglia)	14	12	

superior cervical ganglion of the cat, dog and rabbit, by using bicarbonate-free Ringer containing physostigmine (1:50,000). The dialysates of the extracts were diluted and tested on the isolated frog heart. Table 1 gives the acetylcholine equivalent of extracts in γ per gram of nerve. A few experiments showing the inhibitory effects on the frog heart are illustrated in figure 1.

Control experiments were made with the other acetylcholine-extraction methods, namely, the acid-alcohol method (Engelhart, 1930), trichloracetic acid extraction (Chang and Gaddum, 1933), and the eserine-Ringer-heating method (Loewi and Hellauer, 1938 b); and the extracts were tested on the frog heart, the eserinized *rectus abdominis* of the frog (Chang and Gaddum, 1933), and on the longitudinal muscle of the leech (Minz, 1932). These comparative control titrations showed that the physostigmine-Ringer extract gave higher values than that resulting from either the alcohol or the heating procedure, and was about equal to the trichloracetic acid

extraction. By dialyzing the physostigmine-Ringer extract half the total acetylcholine-content was excluded. This method has the advantage, however, of allowing tests for both positively and negatively acting substances. It was therefore used.

During the process of dialysis no acetylcholine or adrenaline was lost. Control experiments showed that when acetylcholine or adrenaline, diluted to 1:100,000,000 in Ringer's solution containing physostigmine (1:50,000) and dialyzed against equal amounts of physostigmine-Ringer, exactly half the original acetylcholine or adrenaline concentration was found after 3 hours on both sides of the membrane. Such an experiment is illustrated in figure 2.

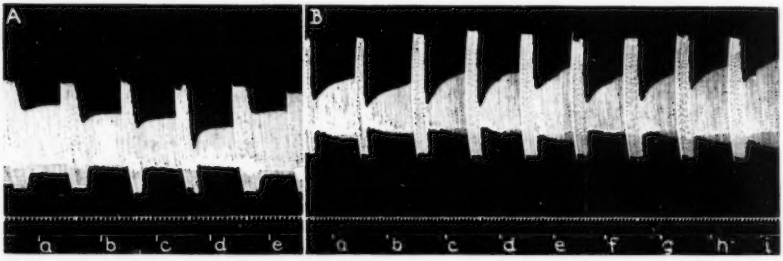

Fig. 1A. Normal frog heart. In this and succeeding records the time signal marks 5-second intervals. Extracts were made with physostigmine-Ringer. At *a*, acetylcholine (a.ch.) 0.001γ; at *b*, a.ch. 0.005γ; at *c*, dialyzed extract (d.e.) of vagus of the cat; at *d*, a.ch. 0.01γ; at *e*, d.e. of cervical sympathetic nerve of the cat.

B. At *a*, d.e. of the sciatic of cat; at *b*, a.ch. 0.01γ; at *c*, d.e. of the cervical vago-sympathetic of dog; at *d*, a.ch. 0.01γ; at *e*, d.e. of the phrenic of cat; at *f*, d.e. of the thoracic chain and ganglia of cat; at *g*, a.ch. 0.008γ; at *h*, d.e. of mesenteric plexus of cat; at *i*, a.ch. 0.005γ.

B. *Extracts of postganglionic sympathetic fibers in physostigmine-Ringer solution.* In order to have pure postganglionic sympathetic fibers for extraction it was necessary to carry out some preliminary sterile operations on the animals used. Preganglionically denervated superior cervical ganglia (with the postganglionic fibers) of the cat and rabbit no longer contain acetylcholine one to two weeks after the operation. They contain an adrenaline-like substance having positive inotropic and chronotropic effects on the hypodynamic frog heart (fig. 3). The acetylcholine content of the superior cervical ganglion remained unaltered when the postganglionic fibers were cut.

In cats and dogs the vagi were cut below the diaphragm. Two weeks later, after complete degeneration of the vagus fibers, the postganglionic sympathetic fibers along the superior mesenteric artery were extracted and were found to contain no acetylcholine but an adrenaline-like substance

(fig. 3). Previous section of the sympathetic postganglionic fibers near the superior mesenteric ganglion, instead of the vagi, led to the disappearance of this substance from the isolated parts of the sympathetic fibers.

C. *Extracts of different nerves in bicarbonate-free Ringer's solution.* If extracts of mixed nerves were made with bicarbonate-free Ringer, without

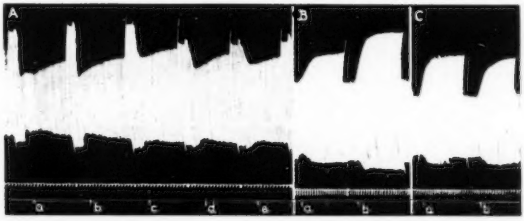

Fig. 2A. Normal frog heart. At *a*, a.ch. fresh, 0.01γ; at *b*, a.ch. 3-hours old, 0.01γ; at *c*, a.ch. 3-hours old, 0.005γ; at *d*, a.ch. 0.01γ dialyzed in physostigmine-Ringer during 3 hours, rest; at *e*, same as *d*, dialysate.

B. Hypodynamic frog heart. At *a*, adrenaline (adr.) 0.005γ fresh; at *b*, adr. 0.01γ fresh.

C. At *a*, dialyzate of 0.01γ adr; at *b*, same, undialyzed remnant.

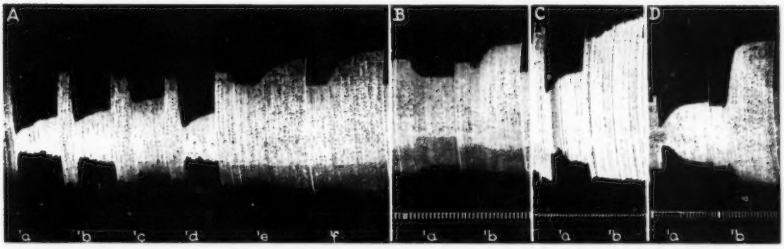

Fig. 3A. Hypodynamic frog heart. Extracts made with physostigmine-Ringer. At *a*, a.ch. 0.01γ; at *b*, a.ch. 0.005γ; at *c*, a.ch. 0.001γ; at *d*, d.e. of normal superior cervical ganglion of cat; at *e*, d.e. of preganglionically denervated (2 weeks) superior cervical ganglion of cat; at *f*, adr. 0.01γ.

B. At *a*, d.e. of normal superior cervical ganglion of rabbit; at *b*, d.e. of preganglionically denervated (2 weeks) superior cervical ganglion of rabbit.

C. At *a*, d.e. of normal mesenteric plexus of dog; at *b*, d.e. of mesenteric plexus of dog (abdominal vagi cut 2 weeks before).

D. At *a*, d.e. of normal mesenteric plexus of cat; at *b*, d.e. of mesenteric plexus of cat (abdominal vagi cut 2 weeks before).

physostigmine, acetylcholine was totally destroyed and only the adrenaline-like substance was present. These experiments showed that an adrenaline-like substance can be extracted from all the nerves examined which contain postganglionic sympathetic fibers. The vagus fibers of the dog contain this substance; those of the cat do not contain it in sufficient

amount to be demonstrable. Evidence of the presence of the substance was found in extracts from the cervical sympathetic ganglia and their fibers, from the superior mesenteric plexus and ganglion and from the sciatic of the dog and cat. The substance was not found in the phrenic nerve. According to Verne (cf. Binet and Minz, 1936) the phrenics of the dog and cat are free of sympathetic fibers. It was also found in the superior mesenteric plexus of the sheep, cow and bull. Table 2 presents the adrenaline equivalent of extracts in γ per gram of nerve. A titration is shown in figure 4.

D. *Some properties of the adrenaline-like substance extracted from the postganglionic sympathetic nerve fibers.* As the extraction method shows, the adrenaline-like substance dialyzes easily through a cellophane membrane. If the test frog heart is washed with Ringer's solution containing ergotoxine (1:100,000) until even high concentrations of adrenaline

TABLE 2

Adrenaline equivalent of extracts in γ per gram nerve

Average values calculated from 20 experiments. The maximal deviation from the average was 30 per cent.

NERVES	CATS	DOGS	RABBITS
Cervical vagus	0	1	0
Cervical sympathetic trunk	0		
Superior cervical ganglia (with postganglionic fibers)	4		3.5
Sciatic	1	0.6	0.8
Phrenic	0	0	0
Superior mesenteric plexus and ganglion	5	4.5	
Thoracic sympathetic chain (including ganglia)	3	3	

(1:10,000,000) are ineffective, the extract of the mesenteric plexus is likewise ineffective (fig. 5). If this extract is boiled for 2 to 3 minutes and tested after cooling it causes no change in the frog heart (fig. 5). Ashing the extract and subsequently making the ash up to the original volume with distilled water results also in a solution without a positive effect. If the solution is made alkaline and oxygen is bubbled through it for 5 hours, the adrenaline-like substance is destroyed. The substance, therefore, is of organic nature, oxydizable and thermolabile, and its action is abolished by ergotoxine.

Discussion. The experiments reported in section A show, as has been reported by others, that the motor, parasympathetic and preganglionic sympathetic nerves and ganglia contain acetylcholine in different amounts.

Two to 4 weeks after section of the preganglionic fibers no detectable amount of acetylcholine was found in the superior cervical ganglia. Brown and Feldberg (1936) showed that the high acetylcholine content of the

normally innervated ganglion is dependent upon the integrity of the preganglionic fibers; normal ganglia yielded the high acetylcholine equivalent of 10 to 20γ per gram, which fell after denervation to 1 to 3γ per gram. Similarly MacIntosh (1938) examined the acetylcholine content of the preganglionically denervated ganglia and he found only 15 per cent of the normal acetylcholine equivalent present 72 hours after the operation. Finally Brücke (1937) found an excess of cholinesterase in the superior

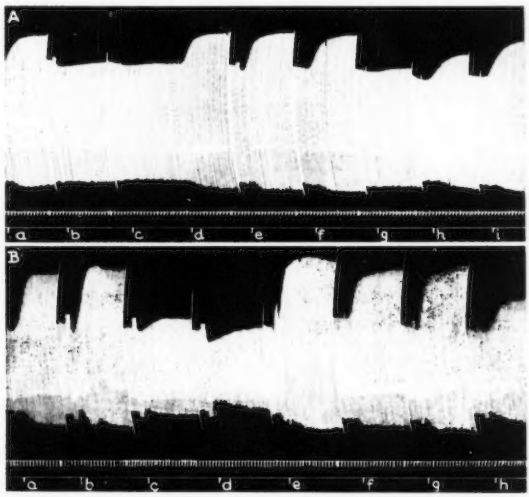

Fig. 4A. Hypodynamic frog heart. Extracts made with bicarbonate-free Ringer. At *a*, d.e. of cervical vagus of dog; at *b*, d.e. of cervical vagus of cat; at *c*, d.e. of phrenic of cat; at *d*, d.e. of superior cervical ganglion of cat; at *e*, adr. 0.01γ; at *f*, d.e. of superior mesenteric plexus and ganglion of cat; at *g*, d.e. of cervical sympathetic nerve of cat; at *h*, d.e. of sciatic of cat; at *i*, d.e. of superior mesenteric plexus of dog.

B. At *a*, adr. 0.01γ; at *b*, d.e. of superior mesenteric plexus of sheep; at *c*, d.e. of cervical vagus of sheep; at *d*, d.e. of cervical vagus of bull; at *e*, d.e. of superior mesenteric plexus of cow; at *f*, adr. 0.01γ; at *g*, d.e. of mesenteric plexus of bull; at *h*, d.e. of superior mesenteric plexus of cow.

cervical ganglion of the cat, which esterase disappeared completely after section of the preganglionic fibers of the ganglion, even before these fibers could have completely degenerated. As shown in table 1 and in figure 1B, *h*, extracts of the superior mesenteric plexus contain acetylcholine. That the presence of this substance is due to the mingling of cholinergic vagal fibers with adrenergic postganglionic sympathetic fibers is demonstrated in experiments reported in section B, figure 3C, *a* and *b*, and D, *a* and *b*. After complete degeneration of the vagal fibers, extract of the

superior mesenteric plexus showed no signs of acetylcholine; only the adrenaline-like substance was manifest.

Since this research was completed Loewi and Hellauer (1938a, b) have shown that the preganglionic sympathetic fibers of cattle contain about six times more acetylcholine than the postganglionic fibers. The presence of acetylcholine in the postganglionic fibers might be explained as due to the admixture of some cholinergic elements. Euler and Gaddum (1931) showed that some cholinergic fibers are mixed with the postganglionic sympathetic fibers of the superior cervical ganglion of dogs.

The experiments outlined in section B present evidence that postganglionic sympathetic fibers contain no acetylcholine, but an adrenaline-like substance. The data from section C prove that an adrenaline-like

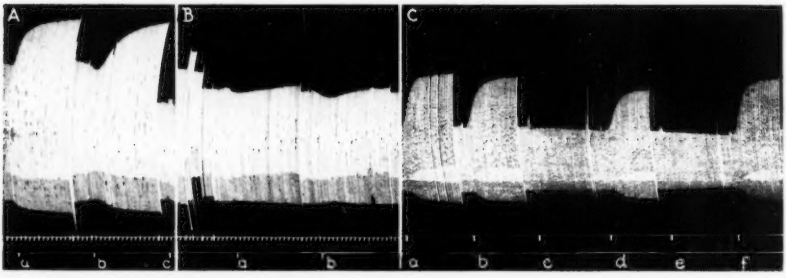

Fig. 5A. Hypodynamic frog heart. At *a*, adr. 0.01γ; at *b*, d.e. of superior mesenteric plexus and ganglion of cat; at *c*, heart washed with ergotoxine-Ringer solution during 3 hours.

B. At *a*, adr. 0.1γ; at *b*, same as A, *b*.

C. At *a*, adr. 0.01γ; at *b*, d.e. of superior mesenteric plexus and ganglion of cat; at *c*, same as *b* after boiling for 3 minutes; at *d*, adr. 0.005γ; at *e*, same as *b* after ashing and redissolved in distilled water; at *f*, same as *b*, 10 times dilution.

substance can be extracted from all the nerves examined which contain postganglionic sympathetic fibers. The few preliminary experiments reported in section D lead to the inference that the properties of the substance extracted from adrenergic nerves are similar to those of adrenaline. The highest content was found in the superior mesenteric plexus, about 4 to 6γ per gram of nerve. This high amount of substance provides the possibility of studying its properties more completely in further experiments.

SUMMARY

1. By using bicarbonate-free Ringer's solution containing physostigmine, extracts were made from different nerves of the cat, dog and rabbit. The extracts were dialyzed and tested on the frog heart. Extracts of

various nerves contain acetylcholine in different amounts (section A, fig. 1 and table 1).

2. Nerve trunks composed only of adrenergic fibers contain no acetylcholine but an adrenaline-like substance (section B, fig. 3).

3. From mixed nerves which contain postganglionic sympathetic fibers an adrenaline-like substance can be extracted (section C, fig. 4 and table 2).

4. The adrenaline-like substance is dialyzable, oxydizable and is destroyed by ashing or by boiling for a few minutes. It has a positive inotropic and chronotropic effect on a hypodynamic frog heart, and this effect is abolished by ergotoxine. The substance has properties similar to adrenaline (section D, fig. 5).

I wish to take this opportunity to express my appreciation to Prof. W. B. Cannon for his many attentions, both personal and scientific, during my stay in the Harvard Physiological Laboratory.

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THE INFLUENCE OF VITAMIN A UPON UREA AND INULIN CLEARANCE IN THE DOG[1]

RAYMOND C. HERRIN AND HENRY J. NICHOLES

From the Department of Physiology, University of Wisconsin, Madison

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In 1937 Herrin, Rabin and Feinstein (1) reported that 5 to 7 hours after the ingestion of butter, there was an increase of about 45 per cent in the urea clearance of dogs. Fat did not seem to be the active principle inasmuch as hydrogenated cottonseed oil was ineffective and the non-saponifiable fraction of butter was effective. The latter preparation would contain, among other substances, the fat soluble vitamins A, D and E. The literature reveals only two references which would indicate that vitamin A had any association with renal physiology. In 1922 Richards and Plant (2) reported that the feeding of liberal quantities of carrots to rabbits resulted in a much better maintenance of urine flow in the course of their experiments performed under anesthesia. In 1927 Cruickshank, Hart and Halpin (3) reported that in chicks showing avitaminosis A, the renal tubules and ureters were filled with urates. This finding has been confirmed by other workers. We have found no reports that vitamins D or E would affect renal function except by the calcification of the kidneys caused by a large excess of vitamin D.

This paper is a demonstration of an effect of vitamin A upon renal excretion as measured by urea and inulin clearances. It is also concerned with the physiological significance and mechanism of the effect of vitamin A upon renal physiology.

EXPERIMENTAL PROCEDURE. Female dogs were used only after they were thoroughly trained to lie quietly, for catheterization and vascular puncture. The bladder was washed with sterile saline before and at the end of every collection period. About 30 minutes before urine collection began the dogs were given by stomach tube 0.5 gram urea and 15 cc. water per kilo of body weight. In many experiments additional water was given but clearances made with a rising rate of urine secretion were avoided or rejected. From 2 to 4 urine collection periods of 22 to 30 minutes' duration were made.

[1] This study received financial support by grants from the Committee on Scientific Research of the American Medical Association and the Wisconsin Alumni Research Foundation.

Clearances designated as post-absorptive were made about 21 hours after the last feeding. The post-prandial clearances were made about 6 hours after feeding in the case of the protein meal and about 9 hours after feeding with fat. All clearances in this report are those made under post-absorptive conditions unless specifically stated otherwise.

In determining the renal excretion at any stage of the experiment we were never satisfied with the clearances of one day alone but conducted them over a period of several days and in most cases we observed the progressive change from one level to another. This paper is based upon the evidence of over 1300 urea clearances and 420 inulin clearances made upon 11 adult and 12 young dogs. When averages are referred to they mean the average of at least 2 to 4 clearances. All clearances are expressed in cubic centimeters of plasma cleared per minute per square meter of body surface and are maximum clearances. The young dogs were 3 to 6 months of age and weighed from 3.3 to 7.0 kgm.

Inulin in both urine and plasma was determined as the increase in reducing substance after acid hydrolysis as outlined by Van Slyke (4). The reducing capacity of the hydrolysate was determined by the Van Slyke and Miller method (5). Urea was determined with urease.

In the preparation of diet I, meat residue was used instead of whole meat because of economy. The meat residue was extracted with ether by a Soxhlet type of extractor for 2 weeks or more. The yeast[2] was similarly extracted with anhydrous ether. Yeast had a very marked effect in improving the palatability of diets 1 and 2. The effect seemed to be exerted too quickly to be explained as due to vitamin B_1. Vitamin D as irradiated ergosterol[3] was added to all diets except diet 6 which was practically free of vitamin D. The diets were as follows:

Diet 1:		Diet 2:	
Meat residue	5000	Meat residue	100
Starch	1080	Starch	50
Wesson oil	250	Yeast	2
Yeast	250	NaCl	2
NaCl	50	$CaCO_3$	0.5
$CaCO_3$	25		

Diet 3:		Diet 4:	
Rolled oats	77	Rolled oats	58
Dried skim milk	15	Meat residue	20
Yeast	3	Cottonseed oil	15
Wesson's salt mixture	3	Yeast	5
		Wesson's salt mixture	2

[2] The yeast was generously contributed by the Premier-Pabst Corporation of Milwaukee, Wisconsin, through the courtesy of Dr. H. Levine.

[3] Contributed by Wisconsin Alumni Research Foundation through the generosity of Dr. H. T. Scott.

Diet 5:	Diet 6:	
Commercial dog biscuit	Rolled oats	72
	Dried skim milk	20
	Casein	5
	$CaCO_3$	1
	$Ca_3(PO_4)_2$	1
	NaCl	1

RESULTS. In the attempt to identify the factor in butter which elevated urea clearance, diets containing large amounts of natural fats as butter and cod liver oil[4] were compared with diets containing purified preparations of vitamins A and D. The daily calorific intake in these 4 dogs (table 1) was the same. The vitamin A intake as crystalline carotene in dog B was

TABLE 1

The effect of various fats upon post-absorptive urea clearance

DOG		UREA CLEARANCE	PER CENT CHANGE IN CLEARANCE
F	Diet 5	31	
	Diet 2 + 150 gm. butter per day	38, 45	+47
K	Diet 5	34, 30	
	Diet 2 + 150 gm. cod liver oil per day	26, 30, 41, 30, 23	+30
B	Diet 5	41	
	Diet 2 + 150 gm. lard 100,000 units A as carotene 9,525 units D as ergosterol	41, 53, 67	+60
P	Diet 5	51, 53	
	Diet 2 + 150 gm. lard 9,525 units D as ergosterol	32, 30, 30, 26, 35	−42

the equivalent of that in the cod liver oil of dog K. The intake of vitamin D in dogs B and P was the equivalent of that in the cod liver oil of K. The per cent change is the maximum.

Comment. The diets furnishing vitamin A produced increases in clearance but the vitamin D diet decreased the clearance and after 43 days the urine was a dull reddish brown color, containing many red and white cells, albumin but no casts. There was no marked deviation in the mean arterial blood pressure. This dog also showed anorexia. The cod liver oil diet also had a deleterious effect even after half of the oil was replaced by the same amount of lard. After 38 days, the clearance was reduced, the urine contained many red cells, 6–8 casts per high power field, and there was

[4] The cod liver oil was contributed by the Abbott Laboratories.

anorexia. It is interesting in this connection to point out that feeding cod liver oil to dairy cows has been reported to reduce the percentage of fat in the milk (6). These experiments suggest that a large dosage of vitamin A affects the magnitude of urea clearance.

The influence of vitamins A and D upon urea clearance in dogs receiving a diet which presumably supplies sufficient vitamin A. Comment. The 50,000 units of vitamin A daily were supplied in 1 cc. of halibut liver oil. The vitamin D intake of dog, W, was equivalent to the vitamin D ingested by dogs S and A in the halibut liver oil. The halibut liver oil and ergosterol were administered over a period of 96 days. The clearances show decreases interspersed with the increases. This fluctuation together with the marked increase in clearance shown by dog S

TABLE 2

The effect of halibut liver oil upon urea clearance

DOG	DIET	POST-ABSORPTIVE UREA CLEARANCE	PER CENT INCREASE
S	Diet 5	30, 22, 23	
	Diet 5 + 50,000 vitamin A in halibut liver oil	30, 30, 26, 33, 36	41
	Diet 5	39, 50, 40	94
	Diet 5	33	
	Diet 5 + N.S.F.*	51, 39	54
A	Diet 5	31, 33, 33, 37, 31	
	Diet 5 + 50,000 vitamin A as in halibut liver oil	43, 50, 37, 54, 50, 64	88
W	Diet 5	31, 34, 30, 33	
	Diet 5 + 850 vitamin D as irradiated ergosterol	33, 33, 38, 33, 31	17

* Non-saponifiable fraction of 1 cc. of halibut liver oil.

after administration of the oil had ceased and the result obtained with a large dose of cod liver oil in table 1 made us consider the possibility of toxic materials in the fish liver oils (6). In the attempt to eliminate the variability we prepared the non-saponifiable fraction (N.S.F.) of halibut oil and fed it in equivalent dosage to dog S and in 26 days the clearance increased. In later experiments the variability of response was largely eliminated and the latent period shortened by reducing the vitamin A dosage to 15,000 to 30,000 daily. It remains a question as to whether this is due to the toxic hypervitaminosis A described by other workers. In the case of dog S the clearance remained elevated for at least 44 days after the halibut liver oil was withdrawn. A third attempt to increase the clearance in dog S failed. Vitamin D had no particular effect in increasing urea clearance in dog W. These data indicate that the addition of a rather

large excess of vitamin A to a diet presumably complete in vitamin A will result after a long latent period in an elevation of urea clearance. These results and those of dog B, table 1, are strikingly different from the concept of vitamin physiology generally held in that an excess of this vitamin exerted an effect. Hitherto, the vitamins manifested themselves only by their absence.

Renal clearance in vitamin A deficiency. Having established some association between the amount of vitamin A in the diet and the magnitude of urea clearance, we attempted to evaluate its physiological significance by establishing avitaminosis A in a number of dogs. Inasmuch as pregnancy makes much greater demands upon renal function, urea clearance was determined during the pregnancy of two dogs, that were fed diets deficient in vitamin A. One of the dogs (J) prior to conception, had been fed diet

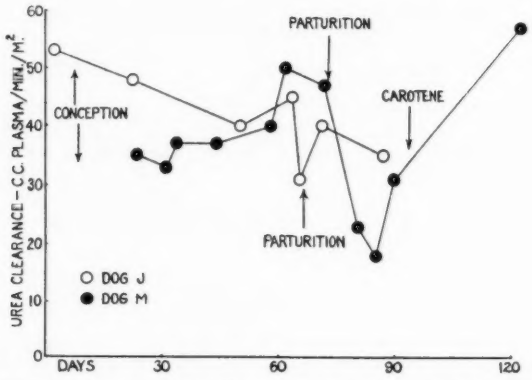

Fig. 1. The effect of vitamin A deficient diets upon urea clearance during pregnancy and lactation.

1 for 6 months with a 42 day period in which she received 7000 units of A as carotene per day. During the entire pregnancy, this dog received 1 cc. of wheat germ oil daily. The other dog had been fed diet 5. We were particularly interested in this experiment after Theobald's report (7) that the administration of vitamins A and D and calcium lactate to pregnant patients seemed to reduce the incidence and severity of the toxemia of pregnancy.

Comment. Referring to figure 1 it is seen that in dog J the clearance during pregnancy decreased to about 42 per cent of its value just before conception. During the last 10 days of pregnancy, the dog showed less activity, some anorexia and appeared sick but there was no vomiting and no marked urinary changes. After parturition she seemed to have very little or no milk. None of the pups survived more than 2 days but autopsy revealed no gross changes.

In dog M, on the other hand, the clearance increased about 44 per cent during the last 10 days of pregnancy. This effect has been reported to be the usual response of normal kidneys in clinical cases of pregnancy (8). Inasmuch as dog J had been previously depleted of much of her stores of vitamin A, the failure of her clearance to show the normal increase during gestation might be attributed to vitamin A deficiency. About 10 days before parturition dog M refused to rise upon her hind legs and simply dragged the hind quarters. If placed upon her hind legs she would stand only a short time. The hind legs showed normal tonus and reflex activity and no spasticity. This motor deficiency might have been due to a failure of some of the descending spinal tracts as has been reported by Mellanby (9) in avitaminosis A in young dogs. On the first day post-partum the dog began walking on her hind legs. The urea clearance decreased to about 50 per cent of normal in the 18 days post-partum. The urine showed little deviation from normal. About this time the pups indicated that they were getting very little milk and when the milk flow ceased the clearance rose to normal which was further increased by carotene. These dogs showed rather severe degrees of vitamin A deficiency but there was nothing which resembled the toxemia in clinical pregnancy except the reduced urea clearance. The wheat germ oil did not seem to affect the urea clearance of dog J and likewise no effect was observed in another experiment with dog C.

Comment. Dogs I, C and P were adult dogs. Dog I had been fed the vitamin A deficient diet for 469 days. In this dog, signs of avitaminosis A were severe. The hairy coat was dry and lusterless, the appetite was moderately impaired, lacrimation was increased and night blindness was very severe. However, there was no xerophthalmia and in regard to general activity in the daylight she showed little deviation from normal. Dog C had been fed the vitamin A deficient diet for 250 days and dog P for 186 days. The signs of avitaminosis A were accordingly much less evident in these dogs. The other dogs of table 3 were placed on the vitamin A deficient diet when about 3 to 4 months of age and weighed from 3.3 to 7.0 kgm.

The development of avitaminosis A in 12 young dogs, 10 of which are listed in table 3, ran a more or less characteristic course. Within 6 to 10 weeks the urea clearance decreased from a level of 36 to 57 to a range of 18 to 33. All of the dogs then showed some amelioration in their signs of avitaminosis A and the urea clearance increased. This recovery was particularly marked with the dogs fed diet 4. The urea clearance in these four dogs returned to 40 to 45. Within a few weeks of this recovery the dogs again showed signs of avitaminosis A and within 3 to 6 months the urea clearances decreased to a level of 21 to 33. In 9 of the 12 dogs of table 3, the urea clearance ranged from 20 to 25. The dogs receiving diet 4 which contained meat residue required a longer time before the clearance dropped to the low level. In avitaminosis A, the dogs are much slower in

starting and do not maintain diuresis nearly as well as when normal. With large amounts of water they are capable of urine flows of over 10 cc./min./M^2 of body surface. The urine showed many epithelial and red blood cells, frequently slight to moderate albuminuria, an occasional cylindroid but no granular or red cell casts. No vitamin A or carotene was detected in the plasma by the Lovibond Tintometer. Near terminus, the urea clearance in two dogs rose to 65 and in a third to 98. The inulin clearance showed a corresponding elevation. In dog R, after the avitaminosis A had become so severe as to result in complete anorexia and a rectal temperature of 108° F., the urea clearance rose to 45. Of the dogs showing

TABLE 3

The effect of avitaminosis A and administration of a large excess of vitamin A upon urea and inulin clearances

DOG	DIET	AVITAMINOSIS A		VITAMIN A	
		Urea	Inulin	Urea	Inulin
I	Diet 1	34	50	35	82
C	Diet 1	39	46	43	87
P	Diet 3	24	54	61	109
R	Diet 3	25	63	44	119
Fn	Diet 3	30	42	51	113
Bn	Diet 3	20	29	42	67
T	Diet 3	23	35	57	123
	Diet 3 + 2 mgm. vitamin B_1	23	32		
II	Diet 4	25	48	35	74
IV	Diet 4	23	41	42	97
I	Diet 4	21	48		
III	Diet 4	25	50		
K1*	Diet 6	25		42	
K2*	Diet 6	33		43	

* These two dogs were loaned for our study by Dr. Frank Kozelka of the Department of Pharmacology.

this terminal increase of clearance dog R was the only one saved by the administration of vitamin A. The urea clearance decreased to 20 within 3 days and then later increased as seen in table 3. Histological sections of kidneys taken from dogs dying of avitaminosis A showed practically no deviation from normal. Such a negative finding has been reported for rats by Wolbach and Howe (10). In some areas the tubular epithelium was more shallow and the proximal tubules seemed to be somewhat dilated. In one adult dog, several calcareous stones about the size of rice kernels were found in both renal pelves. The data of table 3 indicate that even in complete absence of vitamin A, 65 to 70 per cent of the renal capacity remains. This parallels the observation of Baumann (11) that prolonged

growth in rats occurred in the absence of stored or dietary vitamin A. This reduction in clearance is on a basis of altered physiology rather than altered morphology of the kidney.

The administration of carotene, or the non-saponifiable fraction of halibut liver oil or halibut liver oil in doses equivalent to 10,000 to 50,000 units of vitamin A per day produced recovery with the final elevation in clearances seen in table 3. The earliest increase in urea clearance occurred 11 days after the administration of vitamin A began. In dogs IV, II and Fn a marked increase in inulin clearance occurred quite some time before there was an appreciable change in the urea clearance. As expected, the urea clearance of dogs in avitaminosis A can be increased by the ingestion of vitamin A much more quickly than the clearance of dogs receiving a presumably adequate diet. A daily dosage of 50,000 units does not seem to require a shorter latent period of response than a dosage of 20,000 units. Hypervitaminosis A seemed to develop in 3 dogs with carotene in a dosage of 46,600 units and in 2 dogs with halibut liver oil in a dosage of 50,000 units. The dogs showed anorexia and the urea clearance decreased to normal. The variability in the response of the dogs of table 2 was probably due to excessive dosage of vitamin A.

Apparently the clearance cannot be kept permanently elevated with a large dosage of vitamin A. In dog Fn, with 50,000 units of vitamin A daily, the inulin clearance was increased from 42 to a range of 111 to 92 for 87 days. However, by the end of 136 days the inulin clearance had decreased to 78 even though the vitamin A dosage had been maintained. At this stage the high protein diet (diet 1), which had been demonstrated to have no effect on the post-absorptive clearance, was substituted for diet 3. Within 10 days the inulin clearance had risen to 102 and in 19 days to 122. This indicates that although the clearance has returned toward a normal level, under the influence of a large dosage of vitamin A there is a greater renal reserve to meet the need for excretion. When all vitamin A was withdrawn from the diet of dog P (table 3) the urea clearance remained above 50 for at least 44 days.

Comment. The post-prandial clearances were made about 6 hours after the ingestion of 10 grams of casein per kilo of body weight and about 9 hours after 7.5 grams of lard per kilo of body weight. Dog Kl is the only one in which the post-prandial clearances are greater after vitamin A is administered. However, the data do show that in avitaminosis A even of the severity seen in dog I the clearance can be elevated by protein or fat feeding. Also, these data suggest that although vitamin A within certain limits may set the magnitude of renal clearance, the clearance can be temporarily increased by other dietary principles such as protein and fat.

Mechanism of the physiological effect of vitamin A upon urea clearance. The effect of vitamin A upon renal clearance seems to be by way of chang-

ing the magnitude of glomerular filtration. One hundred two simultaneous urea and inulin clearances on 4 adult dogs and 6 young dogs, when the urea clearance was markedly reduced as the result of vitamin A deficiency, gave an average urea clearance/inulin clearance ratio of 0.537. While this value is slightly lower than other workers have found, it approximates the value we have found for normal dogs. After vitamin A administration to the deficient dogs was initiated the increase in inulin clearance generally occurred before that of urea clearance, so that the ratio actually fell below

TABLE 4

Effect of vitamin A upon urea clearance after the ingestion of casein and lard

DOG	AVITAMINOSIS A			VITAMIN A ADMINISTRATION		
	Post-absorptive	Post-prandial per cent change		Post-absorptive	Post-prandial per cent change	
		Casein	Lard		Casein	Lard
K1	29	+45		29	+76	
	37		−62	44	+23	+18
	25	+16	−4	42	+81	
K2	39	+71		34	+44	
	39		+18			
	35	+34	−14	43	+35	
Bn	20	+55		38	+33	
	22		+50	42	+24	0
T	23	+92		37	+48	
	22		+25	37	+38	+11
Fn	33	+17		46		
	32		−3	43	+36	0
I	31	+66		35	+19	
	32		+20			
	29	+70		32	+23	+9

0.50. As the therapy was continued the ratio increased. Sixty-three simultaneous urea and inulin clearances on 3 adult dogs and 6 young dogs, when the clearances were maximally increased by vitamin A, gave an average urea clearance/inulin clearance ratio of 0.514.

The change in magnitude of inulin clearance under the influence of vitamin A might be due to changes in the permeability of the glomerulus. With the large dosage of vitamin A the glomerulus might become more permeable so that instead of the usual 20 per cent of the plasma inulin being extracted by the kidney, a greater amount would be extracted. We

TABLE 5

The excretion of hemoglobin by normal dogs and by dogs with elevated clearances

DOG	EXPERIMENT NUMBER	HEMOGLOBIN CONCENTRATION IN GRAMS PER 100 CC.	
		Plasma	Urine
Fn	1	0.077	0
		0.068	0
	2	0.085	0.071
		0.060	0.061
		0.037	0
	3	0.080	0.18
		0.060	0.08
Br	1	0.072	0
		0.068	0
		0.126	0.083
		0.114	0.066
		0.064	0
	2	0.053	0
		0.055	0
		0.087	0.0275
	3	0.050	0
		0.067	0
		0.074	0
		0.090	Trace
		0.093	Hemoglobinuria
T	1	0.068	0
		0.064	0
	2	0.072	0
		0.070	0
		0.177	0.186
		0.140	0.177
	3	0.062	Trace
		0.039	0
	4	0.055	0
		0.073	0.044
G1 (Normal)	1	0.076	0
		0.083	0.027
K (Normal)	1	0.056	0
		0.050	0
		0.069	0
		0.080	0
		0.090	0.025

have not determined the extraction ratio of inulin but have studied the excretion of hemoglobin as an indication of any changes in permeability. The studies of Bayliss, Kerridge and Russell (12) upon the renal excretion of various proteins by anesthetized dogs indicated that molecular size determined whether the protein would be excreted. Hemoglobin they found to be near the borderline of permeability. Plasma concentrations below 0.15 per cent did not allow excretion but above that level hemoglobinuria occurred. Ottenberg and Fox (13) concluded that in terms of plasma-concentration the renal threshold for hemoglobin in the human subject was very variable.

This was not true in our studies upon normal dogs and upon dogs with elevated clearances as the result of vitamin A administration. The data of table 5 indicate that below a plasma concentration of 0.08 per cent, hemoglobin was not excreted by the kidney but with a concentration only slightly above this value hemoglobin is excreted. Although this value is below that reported by Bayliss for the anesthetized dog it approximates that which we found for 2 normal, conscious dogs. Upon the basis of data in table 5, one may conclude that the effect of vitamin A in elevating inulin clearance is not by increasing glomerular permeability. Serum albumin with a molecular weight slightly greater than hemoglobin is not excreted by the normal kidney. Albuminuria was frequently present in avitaminosis A but was absent when the clearances were elevated by vitamin A administration. These studies indicate that permeability is not a significant factor in the change in clearance produced by vitamin A.

Another possible mechanism by which vitamin A might change inulin clearance would be by an improvement in general bodily condition, with the resultant increase in clearance an indirect effect. Inasmuch as renal function has been demonstrated to be so closely dependent upon the circulation, in 4 dogs we determined hemoglobin, pulse rate and mean arterial blood pressure and in a fifth mean blood pressure when the inulin clearance was low in avitaminosis A and again when the inulin clearance was maximum as a result of vitamin A administration. When the dogs were in a state of avitaminosis A even of severe degree, their physical activity was only slightly below normal, indicating no circulatory failure. Furthermore, as they approached terminus, with the greater likelihood of circulatory impairment, renal clearance actually increased far above the normal. Dr. W. J. Meek very kindly compared the electrocardiograms of 3 of the dogs taken when they were in avitaminosis A and again when the clearances were elevated by vitamin A. He found no difference. Finally, in only one of the five dogs was there a significant increase in mean arterial blood pressure or hemoglobin when the renal clearance had been elevated by the administration of vitamin A. As regards other bodily functions we found no change in gastric secretion but about a third of the dogs did not

show prolonged gastric emptying time. However, there was no correspondence between the degree of avitaminosis A, the low inulin clearance and prolongation of emptying time. In conclusion, it would seem that although impairment of general bodily condition may be partly responsible for the reduced clearance during avitaminosis A, it plays a minor rôle and its improvement is certainly not a factor when the urea and inulin clearances are elevated above normal by the administration of vitamin A.

Discussion. The data contained in this report demonstrate that vitamin A affects the magnitude of urea and inulin clearance. The change in clearance from the low to the high levels in most cases is more than 75 per cent. Of the 20 dogs used in the vitamin A experiments, every animal showed a decrease in clearance when vitamin A was withdrawn and an increase at some time or other when vitamin A was administered. These changes were effected with seven different basal diets which included high protein, high fat and low protein-high carbohydrate. They were effected with butter, cod liver oil, halibut liver oil, the non-saponifiable matter of halibut liver oil and crystalline carotene. They were effected in the case of 2 animals with no change in the amount of food consumed. Vitamin B_1 failed to increase the clearance in avitaminosis A. Vitamin D, in the form of irradiated ergosterol and vitamin E, in wheat germ oil, were without apparent effect upon urea clearance. For these reasons, it is believed that the change in clearance is due specifically to vitamin A.

In regard to the mechanism by which the urea clearance is affected by vitamin A, the inulin clearances are rather conclusive evidence that its basis is a change in the volume of glomerular filtration. The change in inulin clearance is probably not by the anti-diuretic hormone of the pituitary because the evidence indicates that this principle does not affect glomerular filtration (14). For the same reason, neither is vitamin A acting merely as a diuretic. Furthermore, we have observed that there may be a high urine flow with a low clearance and a low urine flow with a high clearance, the change in clearance being due to vitamin A. Pitts (15) reported that administering thyroxin to dogs resulted in an elevation of xylose clearance. The thyroid is probably not responsible for the vitamin A effect upon inulin clearance because there is considerable evidence that vitamin A neutralizes the effect of hyperthyroidism (16). The elevation of inulin clearance in our experiments is probably not by virtue of changes in the permeability of the glomerulus, for two reasons. Albuminuria was frequently observed in avitaminosis A but never when the clearance was high. The excretion of hemoglobin when the clearance was elevated seemed to be no different from that observed in 2 dogs with normal clearances. The following evidence indicates very strongly that general bodily condition is not responsible for the changes in inulin clearance. In avitaminosis A, the dogs show very little impairment in general physical

activity in the daylight; the ingestion of casein or lard increased urea clearance as well as when the deficiency was corrected; as terminus is approached the inulin clearance rises to abnormally high levels; the electrocardiogram showed no deviation from normal and although about a third of the dogs showed prolongation of gastric emptying time, others with clearances just as low had a normal emptying time. Furthermore, in only one of the 5 dogs was there a significant increase in mean arterial blood pressure when the inulin clearance rose from the low to the high level as the result of vitamin A administration.

One aspect of this study arouses speculation. Wolbach and associates (17) found that the histological changes in avitaminosis A were largely confined to epithelial structures. On the basis of these extensive studies Wolbach (18) has postulated that vitamin A may be solely concerned in maintaining an apparatus within cells and not in the physiological processes for which the apparatus is necessary This might explain the long latent period required in our experiments before any change in clearance occurred or before the maximum change was attained. Greater dosage did not seem to shorten this period. Furthermore, although vitamin A deficiency or excess does not seem to alter the structure of the nephron, it apparently does affect its function. The influence of vitamin A upon urea and inulin clearance might be brought about by changing the volume of blood flow to the kidney. Van Slyke and associates (19) found that spontaneous variations in urea clearance of dogs paralleled variations in renal blood flow. The same parallelism was found when the urea clearance was increased by meat feeding (20). Vitamin A might also affect glomerular filtration through an elevation of glomerular capillary pressure by constricting the efferent arteriole as has been suggested for the effect of adrenalin upon the kidney by Richards (21) and Smith (22).

SUMMARY

Diets containing 150 grams of butter or cod liver oil increased the postabsorptive urea clearances of dogs 47 and 30 per cent respectively. Later the clearance with the cod liver oil diet fell below normal, with the presence of red and white cells and casts in the urine. A diet containing 150 grams of lard, supplemented with the vitamin D equivalent of the cod liver oil resulted in no increase in clearance and a final decrease of 42 per cent. Such a diet supplemented with the vitamin D and the vitamin A equivalent of the cod liver oil diet resulted in a 60 per cent increase in urea clearance. Vitamin A was supplied as crystalline carotene.

Supplementing a diet presumably containing sufficient vitamin A with 50,000 units of vitamin A daily in the form of halibut liver oil resulted in a 41 to 94 per cent increase in urea clearance. The earliest increase occurred within a week, the maximum increase occurred after 96 days. In one dog

the clearance remained elevated for 44 days after the supplement was withdrawn. The vitamin D equivalent of the halibut liver oil, as irradiated ergosterol, was without material effect upon urea clearance. The variability in the response was in later experiments largely eliminated and the period required for maximum response shortened by reducing the vitamin A dosage to 20,000 to 30,000 units daily.

In a dog which had been maintained on the deficient diet for 5 months prior to conception the urea clearance decreased about 40 per cent during pregnancy. Parturition appeared to be normal but lactation seemed to be a complete failure. In another dog which was started on the vitamin A deficient diet at the time of conception, the urea clearance in the last 10 days of pregnancy increased 44 per cent. In this period there was paralysis of the hind extremities. After parturition, the paralysis disappeared and the clearance decreased to about 50 per cent of her normal. Lactation at first appeared to be normal but after 10 days it failed and the clearance rose almost to normal. Later, carotene increased the clearance 34 per cent above normal. The picture in these dogs was that of avitaminosis A and not that of toxemia of pregnancy.

Vitamin A deficiency was produced in 12 young and 3 adult dogs. In all dogs the clearances decreased, those receiving meat residue requiring a much longer time. In 10 of the dogs, urea clearance ranged from 20 to 25 cc. No vitamin A was detected in the plasma. The urine contained erythrocytes, leucocytes, epithelial cells and frequently albumin but no casts. Diuresis was much harder to start and maintain. However, large urine flows were possible. Near terminus urea clearance rose to 65 cc. or more. Histological sections of the kidneys from dogs with such an exitus showed practically normal glomeruli and tubules.

Administration of halibut liver oil or its non-saponifiable matter or crystalline carotene corrected the vitamin A deficiency. The dosage varied from 10,000 to 50,000 units of vitamin A daily. A dosage of 15,000 to 30,000 seemed to be most favorable for elevation of inulin clearance. In 9 of the dogs, urea clearance rose above 40 cc. The shortest period for response was 11 days. About 50 days are required before the maximum elevation in clearance is attained. Large dosage did not seem to shorten the period required for maximum response. Hypervitaminosis A seemed to occur in 3 dogs with carotene in a dosage of 46,600 units and in 2 dogs with halibut liver oil in a dosage of 50,000 units. The dogs showed anorexia and the clearance decreased to normal. The clearance cannot be kept permanently elevated with vitamin A. In one dog after 136 days the clearance had almost declined to normal. At this stage, substitution of a high protein diet resulted in a marked elevation of clearance.

Vitamin A changes urea clearance because it changes the volume of glomerular filtration. One hundred and two simultaneous urea and inulin

clearances on 10 dogs, when the clearances were low, gave an average urea clearance/inulin clearance ratio of 0.537. When the clearances were maximum as a result of vitamin A therapy, the average of 63 urea clearance/inulin ratios on 9 dogs was 0.514.

The increase in inulin clearance when vitamin A was administered did not seem to be due to changes in the permeability of the glomerulus. Albuminuria occurred when the clearance was low and not when it was elevated. Hemoglobin excretion by the dogs with clearances increased by vitamin A was no different from that observed in two dogs with the usual clearance. In 4 of 5 dogs the increase in clearance occurred with no significant increase in mean arterial blood pressure. This is convincing evidence that changes in general bodily condition play a minor rôle in elevating urea and inulin clearance from the low level of avitaminosis A to the maximum. It is concluded that vitamin A is involved in the physiology of the kidney and is responsible for at least a part of its excretory capacity.

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Contents of Volume 18, 1938

Tentative Contents of Volume 19, 1939